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Molecular Pathogenetic of Cervical Cancer.

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ABSTRACT

Cervical cancer is a disease in which the cells of the cervix become abnormal and start to grow uncontrollably, forming tumors. It is usually a slow-growing cancer that may not have symptoms but can be found with regular Pap tests (a procedure in which cells are scraped from the cervix and looked at under a microscope). Cervical cancer is almost always caused by human papillomavirus (HPV) infection. P16 were found molecular targets in Cervical cancer. Studies of these protein were done by insilico method. activation of these protein can be induced by anticancer property holding compounds, among these MIS, allin, vinblastine and vincristine and molecular dynamics properties analysed and docked with these targets to determine their binding energies.

Keywords: Cervical cancer, P16 molecular targets, insilico, Molecular dynamics

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INTRODUCTION

Human papillomavirus (HPV) infection is a necessary factor in the development of almost all cases of cervical cancer. HPV vaccines effective against the two strains of HPV that currently cause approximately 70% of cervical cancer have been licensed in the U.S, Canada, Australia and the EU. Since the vaccines only cover some of the cancer causing ("high-risk") types of HPV, women should seek regular Pap smear screening, even after vaccination. [1] Human papillomavirus (HPV) infection with high-risk types has been shown to be a necessary factor in the development of cervical cancer.[8] HPV DNA may be detected in virtually all cases of cervical cancer.[1][8][2] Not all of the causes of cervical cancer are known. Several other contributing factors have been implicated.[9]

Women who have many sexual partners (or who have sex with men who had many other partners) have a greater risk.[10][11] Genital warts are caused by various strains of HPV which are usually not related to cervical cancer. However, it is possible to have multiple strains at the same time, including those that can cause cervical cancer along with those that cause warts. The medically accepted paradigm, officially endorsed by the American Cancer Society and other organizations, is that a patient must have been infected with HPV to develop cervical cancer, and is hence viewed as a sexually transmitted disease but most women infected with high risk HPV will not develop cervical cancer.[16] The naming and histologic classification of cervical carcinoma precursor lesions has changed many times over the 20th century. The World Health Organization classification[19][20] Cervarix, manufactured by GlaxoSmithKline, has been shown to be 92% effective in preventing HPV strains 16 and 18 and is effective for more than four years.[25] Cervarix was approved in the US on 16 October 2009,[26] and in the EU in September 2007, as well as other nations.[27][28] Alliin and Allicin are two important sulfur-containing compounds found in Garlic and Onions and their relatives. Their names are derived from the Latin name of Garlic, *Allium sativum*. Both alliin and allicin are known as "organosulfur" compounds, which mean they are "organic" - contain carbon - and also contain sulfur. Many medicinal organosulfur compounds are found in the *Alliums*, but Garlic contains the highest concentration of them (54).

Extracts of Vinca have significant anticancer activity against numerous cell types. The greatest activity is seen against multi-drug resistant tumor types which suggest that there are compounds in *Vinca rosea* that are synergistic or additive with anti-neoplastic elements by inhibiting resistance to them. This library includes vincalkebostine (vinblastine),²² - oxovincaleukoblastine (vineristine), reserpine, vincamine, vincristine, vinblastine, leurocristine, ajmalicine.

MATERIALS AND METHODS

Databases:

1. Genbank:

Databank of genetic sequences operated by a division of the NATIONAL INSTITUTE OF HEALTH.

2. Protein Data Bank:

A Protein Data Bank (PDB) is a database, which has a collection of protein structure [10]

3. Genecard:

Genecard is a database, which has collection of information about the genes.

Tools :

1. Arguslab:

A molecular modeling, graphics, and drug design. Arguslab offers geometry optimisation using the MNDO, AM1 or PM3 semiempirical levels, as well as single point calculations using these, though the range of elements covered is much less [11]. There are also single point semiempirical calculations using Extended

Huckel (for a bigger element coverage) or ZINDO (for excited states for UV/visible absorption prediction). Arguslab has good facilities for calculating electron density or orbital surfaces at the semiempirical levels, and displaying them. Arguslab writes its own format of molecule file, .xml, but it can also write .xyz files for input to other programs, e.g. molden. It creates (and leaves behind) a lot of temporary files, which need to be managed.

2. Hyperchem:

HyperChem is a Sophisticated molecular modeling environment that is known for its quality, flexibility and ease of use. Uniting 3D visualization and animation with quantum chemical calculation, molecular mechanics and dynamics [12]. The QSAR properties were determined using this software. Binding affinity of the ligands used for the present study were analysed using QSAR properties.

1. PRODRG Server:

PRODRG server is a tool for high-throughput crystallography analyzer for protein-ligand complexes [13].

2. CASTP:

CastP server is a tool for predicting the protein pockets where the ligands can bind.

3. Molegro Virtual Docker:

Molegro Virtual Docker (MVD) is an integrated environment for studying and predicting ligands interact with macromolecules [14]. The identification of ligand binding modes is done by iteratively evaluating the ligand conformations and estimating the energy of their interactions with the macromolecules.

4. Yasara:

Yet Another Scientific Artificial Reality Application (YASARA) is a molecular-graphics, -modeling and -simulation program for Windows and Linux developed in 1993.

5. Methodology

1. The given query term P16 was searched in Genecard and details about that were shown.
2. The PDB files of the ligand molecules (Combinatorial Library) were built by Hyperchem software [15].
3. The Molecular Dynamics and Energy Minimization of the ligand molecules were done in Argus Lab by adjusting the corresponding parameters.
4. QSAR properties were analyzed in Hyperchem and ProDRG server.
5. The cavity of the targets was determined by CastP.
6. In Molegro Virtual Docker the selected targets and ligands were docked.
7. The best hit of the result was selected based on the highest score, energy and the RMSD values.
8. Results of the best hit were selected and downloaded.
9. Viewed by using visualization program - YASARA

RESULTS AND DISCUSSION

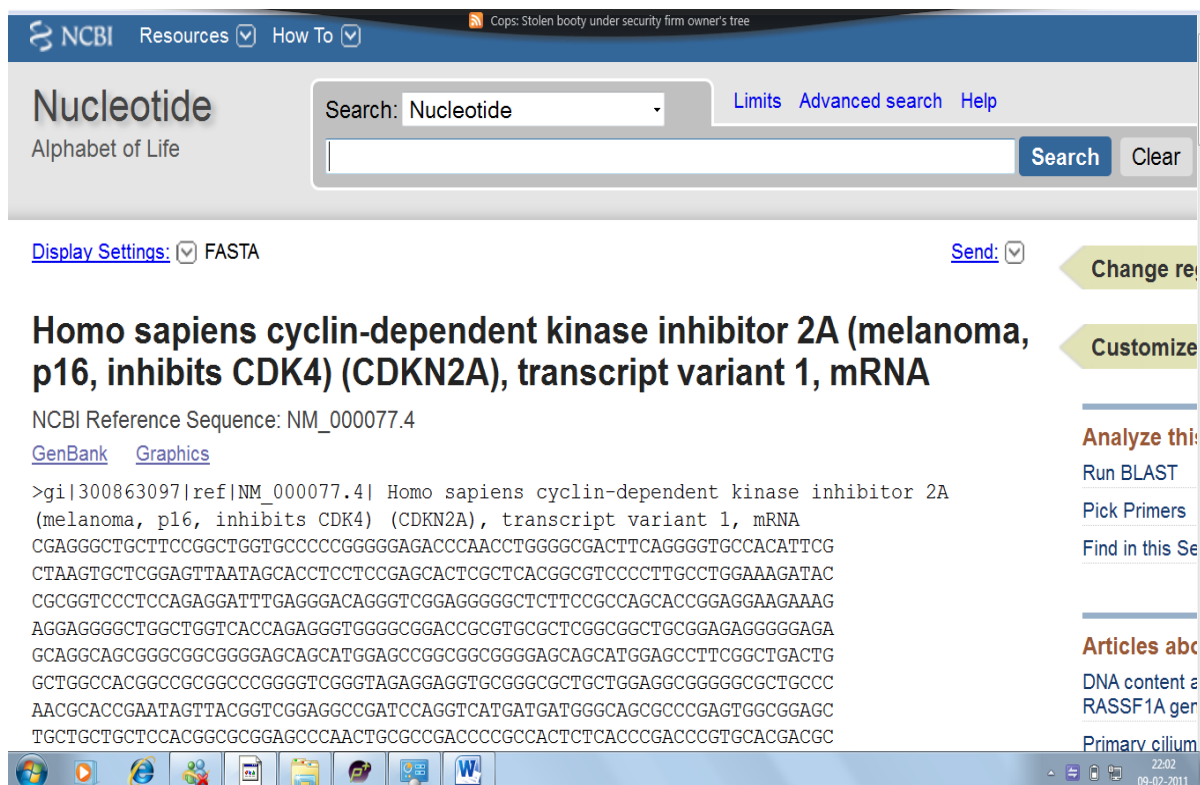
Gene Card Database



	Symbol	Description	Category	GIFs	GC id	Score
1	CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	protein-coding	70	GC09M021957	49.93
2	ARPC5	actin related protein 2/3 complex, subunit 5, 16kDa	protein-coding	55	GC01M183592	14.45
3	CDK4	cyclin-dependent kinase 4	protein-coding	72	GC12M058142	8.24
4	TP53	tumor protein p53	protein-coding	80	GC17M007565	8.17
5	RB1	retinoblastoma 1	protein-coding	70	GC13P048877	7.76
6	CDKN2B	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	protein-coding	62	GC09M021992	7.21
7	OFD1P16Y	OFD1 pseudogene 16, Y-linked	pseudogene	10	GC0YM023958	6.05
8	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	protein-coding	72	GC08P128748	4.95
9	EGFR	epidermal growth factor receptor	protein-coding	76	GC07P055054	4.71
10	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	protein-coding	62	GC12M025358	4.24
11	MC1R	melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)	protein-coding	63	GC16P089985	4.11
12	MTAP	methylthioadenosine phosphorylase	protein-coding	66	GC09P021792	3.84
13	WHSC1	Wolf-Hirschhorn syndrome candidate 1	protein-coding	62	GC04P001840	3.78
14	S100P	S100 calcium binding protein P	protein-coding	54	GC04P006695	3.18

From Gene Card Database, the targets P16 details were taken to analyse.

NCBI - GENE:

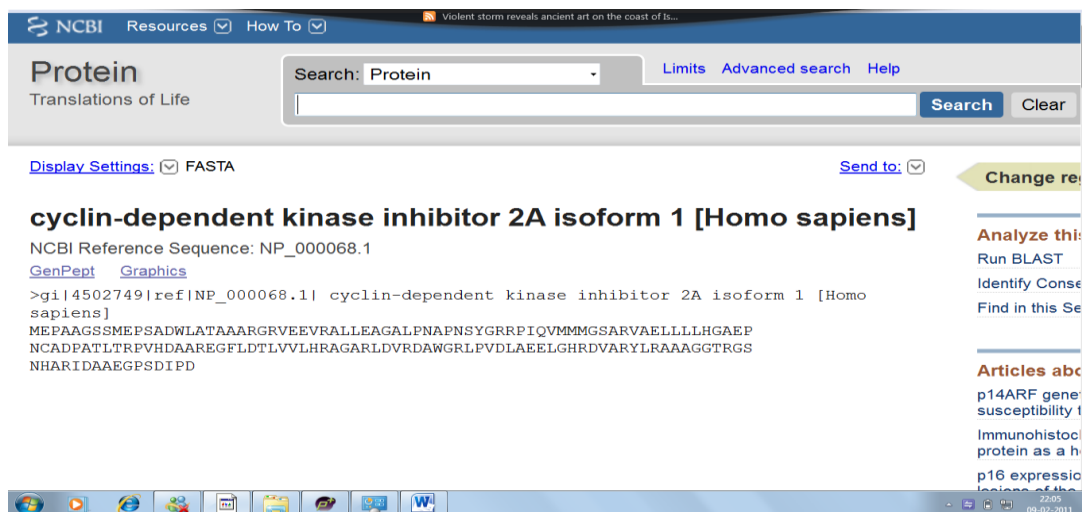


NCBI Reference Sequence: NM_000077.4

GenBank Graphics

```
>gi|300863097|ref|NM_000077.4| Homo sapiens cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) (CDKN2A), transcript variant 1, mRNA
CGAGGGCTGCTTCCGGCTGGTCCCCCGGGGAGACCAACCTGGGGCGACTTCAGGGGTGCCACATTTCG
CTAAGTGTCTCGGAGTTAATAGCACCTCCTCCGAGCACTCGCTCACGGCGTCCCCTTGCCTGGAAAGATAC
CGCGGTCCCTCCAGAGGATTTGAGGGACAGGGTTCGGAGGGGGCTCTTCCGCCAGCACCGGAGGAAGAAAG
AGGAGGGGCTGGCTGGTACCAGAGGGTGGGGCGGACCGCGTGCCTCGCGGCTGCGGAGAGGGGGAGA
GCAGGCAGCGGGCGGGGAGCAGCATGGAGCCGGCGGGGAGCAGCATGGAGCCTTCGGCTGACTG
GCTGGCCACGGCCGGCCCGGGGTCCGGTAGAGGAGGTGCGGGCGCTGCTGGAGCGGGGGCGCTGCC
AACGCACCGAATAGTTACGGTCGGAGGCCGATCCAGGTATGATGATGGGCAGCCCGAGTGGCGGAGC
TGCTGCTGCTCCACGGCGGGAGCCCACTGCGCCGACCCCGCCACTCTCACCCGACCGGTGCACGAGCGC
```

PROTEIN:

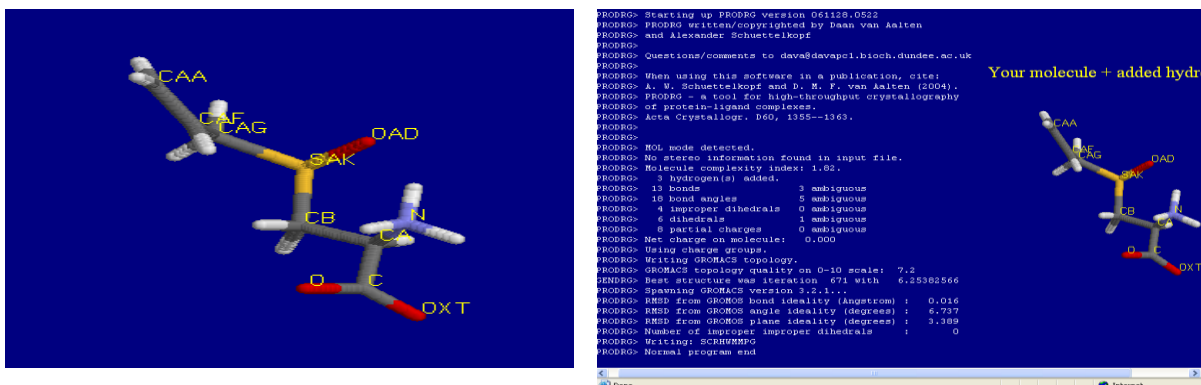


The Sequences were obtained in FASTA format.

Thus the information of P16 was retrieved. There were totally 1267bp nucleotide residues and 156 amino acid residues for P16. The results in Fasta format were saved to the local hard disk for future use.

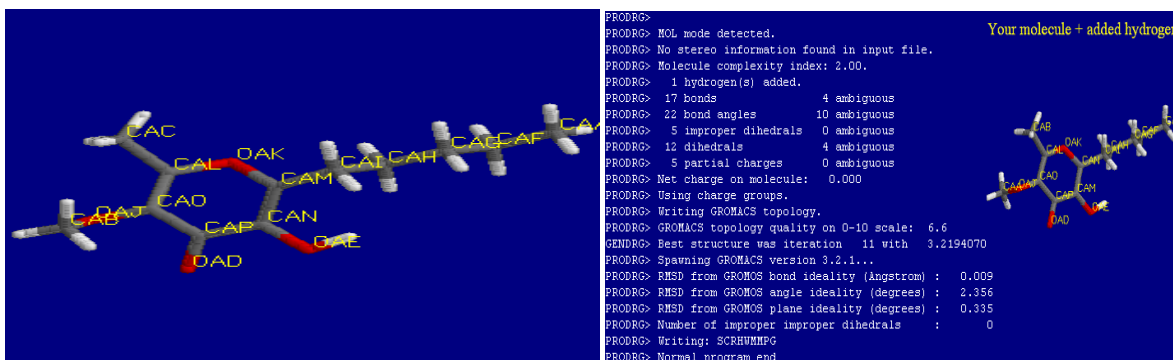
SELECTED COMPOUND FROM LIGAN D LIBRARY [16]

MIS:



The binding efficiency of MIS was calculated as RMSD bond ideality: 0.016
RMSD angle ideality: 6.637

ALLIIN:



The binding efficiency of ALLIIN was calculated as RMSD bond ideality: 0.009
 RMSD angle ideality: 2.356

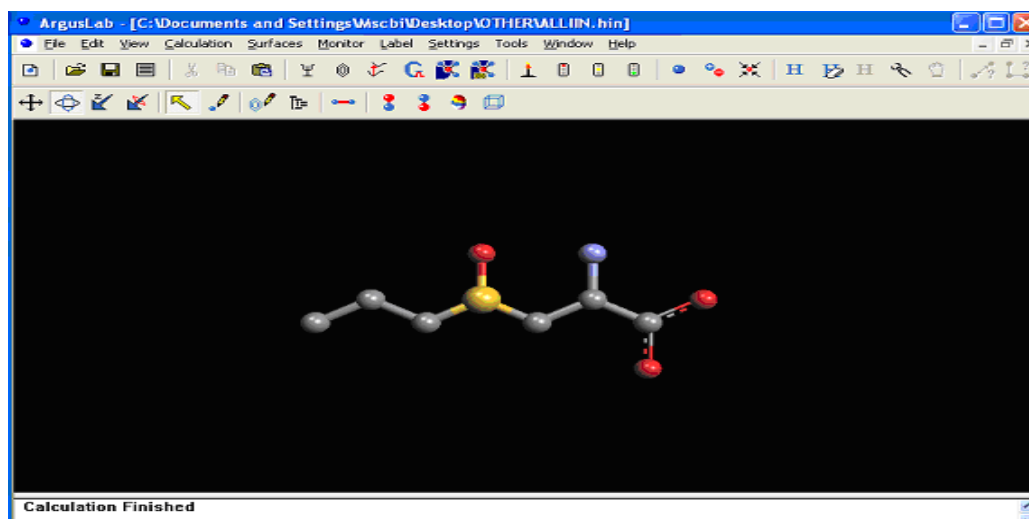
QSAR FOR MIS, ALLIIN, VINCRISTINE AND VINBLASTINE.

A	B	C	D	E
CHEMICAL COMPOUND	PARTIAL CHARGES	SURFACE AREA(APP)	SURFACE AREA(GRID)	VOLUME
MIS	0.00e	350.49 A2	342.59 A2	501.25 A3
ALLIIN	0.00e	377.65 A2	334.46 A2	489.79 A3
VINCABLASTINE	0.00e	827.90 A2	908.95 A2	1773.17 A3
VINCRISTINE	0.00e	827.94 A2	908.96 A2	1773.17 A3

E	F	G	H	I	J
VOLUME	HYDRATION ENERGY	LOG P	REFRACTIVITY	POLARIZABILITY	MASS
501.25 A3	-3.03 Kcal/mol	0.86	35.14 A3	12.43 A3	176.21amu
489.79 A3	-7.35 Kcal/mol	1.08	30.94 A3	9.22 A3	166.13amu
1773.17 A3	1.34 Kcal/mol	5.39	167.99 A3	60.74 A3	761.60amu
1773.17 A3	1.34 Kcal/mol	5.39	167.99 A3	60.74 A3	761.60amu

THE GEOMETRY OPTIMIZATION & MOLECULAR DYNAMICS RESULTS OF LIGANDS WERE OBTAINED FROM ARGUS LAB

MIS:



PM3 - QUANTUM MECHANICS CALCULATION FOR MIS [18]

System Type Quantum Mechanical
 Hamiltonian PM3 (NDDO)
 SCF Type UHF
 Run Type SCF
 Atoms 11
 Electrons 53
 Alpha 27
 Beta 26
 Multiplicity 2
 Water Model SPCE
 Coordinates angstroms
 Basis set Minimal Valence Basis as STO 6G
 Max. SCF cycles 200
 SCF convergence 1.5936e-013 au. for energy
 PM3 param file C:\Program Files\ArgusLab\params\pm3.prm
 SCF saved every 1000 cycles

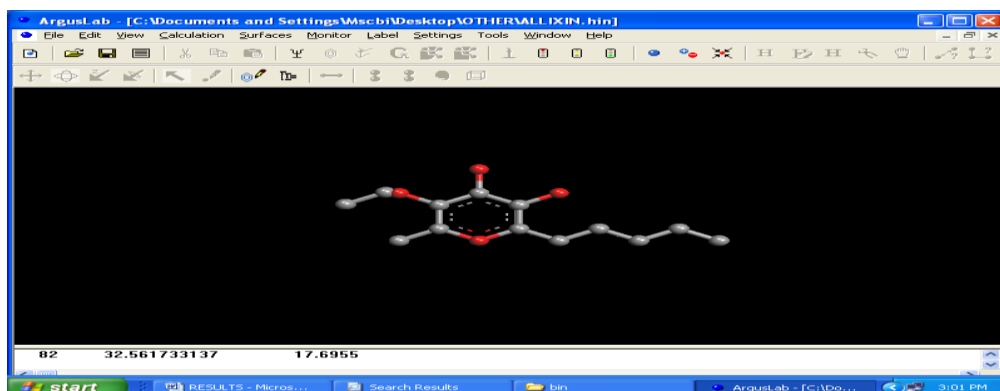
Two-electron integrals

buffer size 1000
 storage random list in core
 Property integrals one center
 Dipole integrals length operator

Input Atomic Information

```
*****
1 C  1.856000 -7.481000  0.000000
2 C  3.155000 -6.731000  0.000000
3 C  4.454000 -7.481000  0.000000
4 S  5.753000 -6.731000  0.000000
5 C  7.052000 -7.481000  0.000000
6 C  8.351000 -6.731000  0.000000
7 C  9.650000 -7.481000  0.000000
8 O 10.950000 -6.731000  0.000000
9 O  9.650000 -8.981000  0.000000
10 O 5.753000 -5.231000  0.000000
11 N 8.351000 -5.231000  0.000000
```

ALLIIN:



PM3 - QUANTUM MECHANICS CALCULATION FOR ALLIIN:

```
System Type            Quantum Mechanical
Hamiltonian            PM3 (NDDO)
SCF Type                RHF
Run Type                SCF
Atoms                   17
Electrons               76
Water Model            SPCE
Coordinates            angstroms
Basis set               Minimal Valence Basis as STO 6G
Max. SCF cycles        200
SCF convergence        1.5936e-013 au. for energy
PM3 param file         C:\Program Files\ArgusLab\params\pm3.prm
SCF saved every        1000 cycles
```

Two-electron integrals [19]

buffer size 1000
 storage random list in core
 Property integrals one center
 Dipole integrals length operator

Input Atomic Information

```

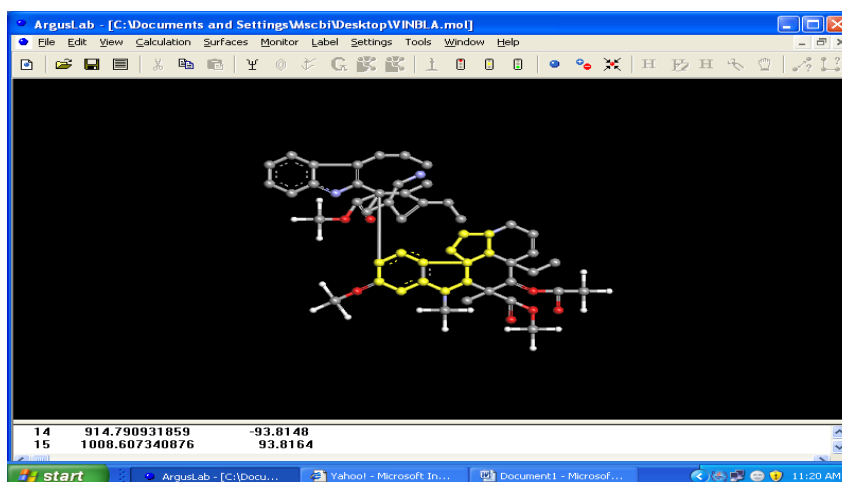
*****
  1 C  7.833000 -8.091000  0.000000
  2 C  9.132000 -8.841000  0.000000
  3 C  9.133000 -10.339000  0.000000
  4 O  7.833000 -11.091000  0.000000
  5 C  6.534000 -10.341000  0.000000
  6 C  6.535000 -8.839000  0.000000
  7 O  7.833000 -6.591000  0.000000
  8 O 10.431000 -8.091000  0.000000
  9 O  5.236000 -8.089000  0.000000
 10 C  5.235000 -11.091000  0.000000
 11 C 10.432000 -11.089000  0.000000
 12 C 11.731000 -10.339000  0.000000
 13 C 13.030000 -11.089000  0.000000
 14 C 14.329000 -10.339000  0.000000
 15 C 15.628000 -11.089000  0.000000
 16 C  4.796000 -8.034000  0.000000
 17 C  3.497000 -8.784000  0.000000
  
```

Atomic spin densities

```

*****
 75 H  0.0000
S2 operator
*****
exact      0.750000
calculated 0.750000
Properties elapsed time 0 sec.
Total Elapsed Time 6 min. 6 sec.
  
```

VINBLASTINE:



PM3 - QUANTUM MECHANICS CALCULATION FOR VINBLASTIN:

System Type	Quantum Mechanical
Hamiltonian	PM3 (NDDO)
SCF Type	UHF
Run Type	SCF
Atoms	75
Electrons	273
Alpha	137



Beta 136
Multiplicity 2
Water Model SPCE
Coordinates angstroms
Basis set Minimal Valence Basis as STO 6G
Max. SCF cycles 200
SCF convergence 1.5936e-013 au. for energy
PM3 param file C:\Program Files\ArgusLab\params\pm3.prm
SCF saved every 1000 cycles

Two-electron integrals

buffer size 1000
storage random list in core
Property integrals one center
Dipole integrals length operator

Input Atomic Information

1	C	2.419000	-2.306000	0.000000
2	C	3.718000	-1.556000	0.000000
3	C	5.016000	-2.305000	0.000000
4	C	5.017000	-3.806900	0.000000
5	C	3.718000	-4.557000	0.000000
6	C	2.418000	-3.805000	0.000000
7	C	7.615000	-2.305000	0.000000
8	C	7.615000	-3.805000	0.000000
9	N	6.314000	-4.554900	0.000000
10	C	8.913900	-1.555000	0.000000
11	C	10.413900	-1.555000	0.000000
12	C	11.712900	-2.305000	0.000000
13	C	8.913900	-4.554900	0.000000
14	C	10.413900	-4.554900	0.000000
15	C	11.712900	-3.805000	0.000000
16	C	7.594000	-5.230900	0.000000
17	C	10.012900	-3.825000	0.000000
18	N	11.311900	-3.075000	0.000000
19	C	8.156000	-6.019000	0.000000
20	C	9.455000	-5.269000	0.000000
21	O	6.865000	-6.604000	0.000000
22	O	8.365000	-6.604000	0.000000
23	C	11.474900	-5.288000	0.000000
24	C	10.205000	-6.567900	0.000000
25	C	12.974900	-5.288000	0.000000
26	C	13.724900	-6.586900	0.000000
27	C	8.887900	-10.012900	0.000000
28	C	10.186900	-9.262900	0.000000
29	C	11.484900	-10.010900	0.000000
30	C	11.486000	-11.512900	0.000000
31	C	10.186900	-12.262900	0.000000
32	C	8.887000	-11.510900	0.000000
33	C	14.083900	-10.010900	0.000000
34	C	14.083900	-11.510900	0.000000
35	N	12.782000	-12.260900	0.000000
36	C	15.382900	-9.260900	0.000000
37	C	16.681000	-10.009900	0.000000
38	C	16.681900	-11.512000	0.000000
39	C	15.382900	-12.262000	0.000000

```

40 N 15.382900 -7.761000 0.000000
41 C 16.679900 -7.011000 0.000000
42 C 17.981000 -7.761000 0.000000
43 C 17.981000 -9.260900 0.000000
44 C 13.882900 -7.761000 0.000000
45 C 13.132900 -9.060000 0.000000
46 C 17.980000 -10.759900 0.000000
47 C 19.278900 -10.009900 0.000000
48 C 14.083900 -13.012000 0.000000
49 C 12.782000 -13.760900 0.000000
50 O 7.588000 -12.260900 0.000000
51 C 16.681900 -13.012000 0.000000
52 O 16.681900 -14.512000 0.000000
53 O 17.981000 -13.762000 0.000000
54 C 17.981000 -15.262000 0.000000
55 O 17.981000 -12.262000 0.000000
56 C 19.481000 -12.262000 0.000000
57 C 20.981000 -12.262000 0.000000
58 O 19.481000 -13.762000 0.000000
59 C 6.288900 -13.010900 0.000000
60 C 5.365000 -6.604000 0.000000
61 H 20.981000 -10.762000 0.000000
62 H 22.481000 -12.262000 0.000000
63 H 20.981000 -13.762000 0.000000
64 H 19.481000 -15.262000 0.000000
65 H 16.481000 -15.262000 0.000000
66 H 17.981000 -16.762000 0.000000
67 H 5.538900 -11.711900 0.000000
68 H 7.038900 -14.310000 0.000000
69 H 4.990000 -13.760900 0.000000
70 H 5.365000 -8.103900 0.000000
71 H 5.365000 -5.104000 0.000000
72 H 3.865000 -6.604000 0.000000
73 H 11.282000 -13.760900 0.000000
74 H 14.282000 -13.760900 0.000000
75 H 12.782000 -15.260900 0.000000

```

Atomic spin densities

75 H 0.0000

S2 operator

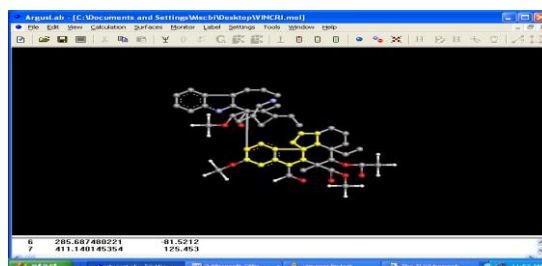
exact 0.750000

calculated 0.750000

Properties elapsed time 0 sec.

Total Elapsed Time 6 min. 6 sec.

VINCRIStINE:





PM3 - QUANTUM MECHANICS CALCULATION FOR VINCRIStINE:

System Type Quantum Mechanical
Hamiltonian PM3 (NDDO)
SCF Type UHF
Run Type SCF
Atoms 74
Electrons 277
Alpha 139
Beta 138
Multiplicity 2
Water Model SPCE
Coordinates angstroms
Basis set Minimal Valence Basis as STO 6G
Max. SCF cycles 200
SCF convergence 1.5936e-013 au. for energy
PM3 param file C:\Program Files\ArgusLab\params\pm3.prm
SCF saved every 1000 cycles

Two-electron integrals

buffer size 1000
storage random list in core
Property integrals one center
Dipole integrals length operator

Input Atomic Information

1	C	2.419000	-2.306000	0.000000
2	C	3.718000	-1.556000	0.000000
3	C	5.016000	-2.305000	0.000000
4	C	5.017000	-3.806900	0.000000
5	C	3.718000	-4.557000	0.000000
6	C	2.418000	-3.805000	0.000000
7	C	7.615000	-2.305000	0.000000
8	C	7.615000	-3.805000	0.000000
9	N	6.314000	-4.554900	0.000000
10	C	8.913900	-1.555000	0.000000
11	C	10.413900	-1.555000	0.000000
12	C	11.712900	-2.305000	0.000000
13	C	8.913900	-4.554900	0.000000
14	C	10.413900	-4.554900	0.000000
15	C	11.712900	-3.805000	0.000000
16	C	7.594000	-5.230900	0.000000
17	C	10.012900	-3.825000	0.000000
18	N	11.311900	-3.075000	0.000000
19	C	8.156000	-6.019000	0.000000
20	C	9.455000	-5.269000	0.000000
21	O	6.865000	-6.604000	0.000000
22	O	8.365000	-6.604000	0.000000
23	C	11.474900	-5.288000	0.000000
24	C	10.205000	-6.567900	0.000000
25	C	12.974900	-5.288000	0.000000
26	C	13.724900	-6.586900	0.000000
27	C	8.887900	-10.012900	0.000000
28	C	10.186900	-9.262900	0.000000
29	C	11.484900	-10.010900	0.000000
30	C	11.486000	-11.512900	0.000000



31 C 10.186900 -12.262900 0.000000
32 C 8.887000 -11.510900 0.000000
33 C 14.083900 -10.010900 0.000000
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35 N 12.782000 -12.260900 0.000000
36 C 15.382900 -9.260900 0.000000
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38 C 16.681900 -11.512000 0.000000
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41 C 16.679900 -7.011000 0.000000
42 C 17.981000 -7.761000 0.000000
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46 C 17.980000 -10.759900 0.000000
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48 C 14.083900 -13.012000 0.000000
49 C 12.782000 -13.760900 0.000000
50 O 7.588000 -12.260900 0.000000
51 C 16.681900 -13.012000 0.000000
52 O 16.681900 -14.512000 0.000000
53 O 17.981000 -13.762000 0.000000
54 C 17.981000 -15.262000 0.000000
55 O 17.981000 -12.262000 0.000000
56 C 19.481000 -12.262000 0.000000
57 C 20.981000 -12.262000 0.000000
58 O 19.481000 -13.762000 0.000000
59 C 6.288900 -13.010900 0.000000
60 C 5.365000 -6.604000 0.000000
61 O 14.080900 -14.510900 0.000000
62 H 20.981000 -10.762000 0.000000
63 H 22.481000 -12.262000 0.000000
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65 H 19.481000 -15.262000 0.000000
66 H 16.481000 -15.262000 0.000000
67 H 17.981000 -16.762000 0.000000
68 H 5.538900 -11.711900 0.000000
69 H 7.038900 -14.310000 0.000000
70 H 4.990000 -13.760900 0.000000
71 H 5.365000 -8.103900 0.000000
72 H 5.365000 -5.104000 0.000000
73 H 3.865000 -6.604000 0.000000
74 H 11.483000 -14.510900 0.000000

Atomic spin densities

74 H 0.0001

S2 operator

exact 0.750000

calculated 0.750000

Properties elapsed time 1 sec.

Total Elapsed Time 6 min. 23 sec.



CONCLUSION

The study of the structural & functional properties of the target P16 was done by *insilico* method. These targets are responsible for the molecular pathogenetic state of Cervical cancer [19]. Inhibitor of P16 such as MIS, Allin, Vinblastine and Vincristine were found to be the best drug for anticancer activity of cervical cancer and can be used as an effective anti- Cervical cancer drugs after performing *invitro* experiments in future.

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