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# Design and discovery of novel therapeutic drugs against *Helicobacter pylori* gastroduodenal cancer by Insilico approach

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**ABSTRACT**

*Helicobacter pylori* is a notorious human and veterinary pathogen responsible for Gastroduodenal cancer due to the epithelial cell signaling mediated by Cytotoxin-Associated Gene A (Cag A). The 3D structure of Cag A is not yet known, such information are crucial for understanding the drug binding mechanism and development of novel agonists. In this study we modeled a 3D structure of Cag A protein by X-ray crystal structure of Dihydroorotate Dehydrogenase (PDB ID 2B4G: A) of *Trypanosome brucii* as the template. The RMSD value of modeled structure was found to be 1.2 $A^{o}$ and steriochemical validation shows 89. 5%, almost all residues are allotted region of Ramchandran plot. Further validation was done by various molecular dynamic emperical force fields. Overall quality factor of model identified to be 93.06; error values of individual residue are negligible. Molecular docking was performed to design and optimize new potential drugs against the disease by *in silico* approach. Our study concluded that plant alkaloids such as Novebine, Taxotere, Taxol and Vinblastin are better drugs than antibiotics as it shows better binding energy with the modeled protein. As the best, Novelbine could be used as suitable drug of choice against gastroduodenal cancer.

**Key words:** *Helicobacter pylori,* Gastroduodenal cancer, CagA*, in silico* modeling, docking, Novelbine

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## INTRODUCTION

Persistent *Helicobacter pylori* colonization of the human stomach is a risk factor for several diseases, including non-cardiac gastric adenocarcinoma, gastric lymphoma, peptic ulceration and MALT lymphoma [1,2] Strains of *H.pylori* are grouped into two broad families tentatively named type I and type II, based on whether they express or not the Vacuolating Cytotoxin (VacA) and the CagA antigen (Cytotoxin-Associated gene A). An increasing body of evidence has shown that patients with duodenitis, duodenal ulcers, and gastric tumors are most often infected by type I strains, which suggests that CagA and the co expressed cytotoxin play a role in its pathogenicity [3] .

The CagA gene was found to be part of a pathogenicity island (PAI), the Cag PAI, a horizontally transferred 40Kb gene fragment containing 27 genes [4] This PAI encodes for virulence factors unique to *H.pylori* strains with enhanced virulence, which suggests that the acquisition of this region is an important event in the evolution of *H.pylori* and marks the differentiation of a more virulent type of bacterium within this genus. The CagA gene of *H.pylori* is assumed as partially responsible for eliciting signaling mechanisms that lead to the development of gastric adenocarcinoma. Some epidemiological studies have demonstrated roles of CagA positive *H.pylori* in the development of atrophic gastritis, peptic-ulcer disease and gastric carcinoma [5] CagA interacts with epithelial cells and mediates complex signaling pathway resulting gastrodueodenal ulcer (Fig.1) *H. pylori* cells with intact Cag islands, including an active type IV secretion system, possess a pilus composed of CagY protein. The CagA product is injected into the cytoplasm of the host cell, where tyrosine (Y) residues near its COOH-terminus are phosphorylated. Phosphotyrosine- CagA interacts with several major signal-transduction pathways in the host cell affecting phenotypes including cell morphology, proliferation and apoptosis. [6]

The injected CagA protein also interacts with Grb2 and activates the Ras/MEK/ERK pathway, leading to the phenotypes of cell scattering (in AGS cells) and proliferation (in MDCK cells) .Tyrosine-phosphorylated CagA binds and activates C-terminal Src kinase (CSK) via its SH2 domain, which in turn inactivates the Src family of protein-tyrosine kinases. Since this signaling may induce apoptosis, the Csk pathway may attenuate the other CagA interactions [7]. By inactivating Src, tyrosine-phosphorylated CagA induces dephosphorylation of cortactin, which then co localizes with filamentous actin (F-actin), in the tip and base of hummingbird protrusions [8]. Thus, the *H.pylori* CagA protein interacts with several of the major signal-transduction pathways present in epithelial cells. *H.pylori* cells with the *Cag Island* deleted have remarkably little interaction with AGS cells in tissue culture [9]; conversely, the CagA apparatus promotes anti-apoptotic pathways, which may aid persistence by slowing turnover of the epithelial cells to which they are attached.

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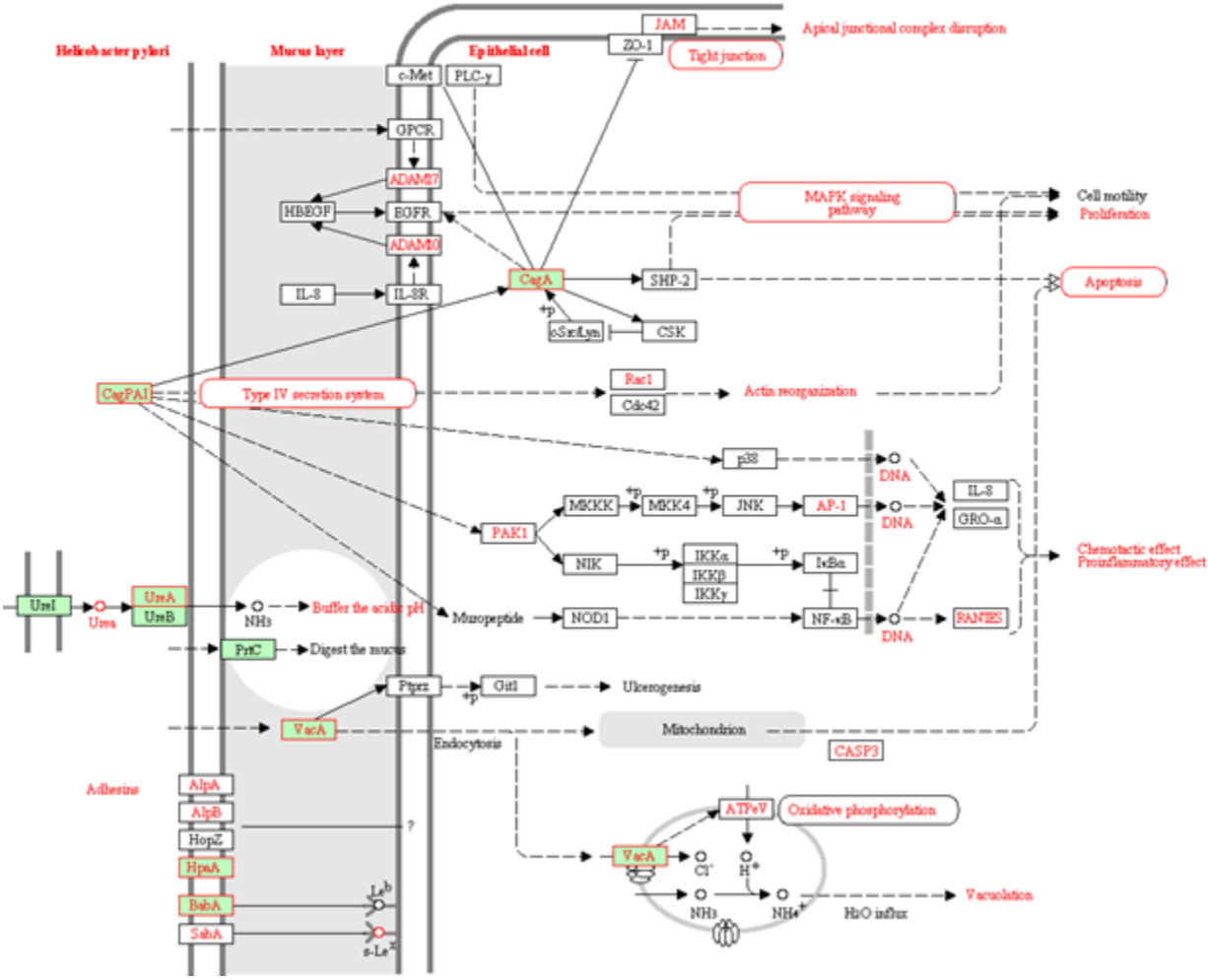


**Fig.1: Epithelial cell signaling in *Helicobacter pylori* mediated by CagPAI (retrieved from KEGG database)**

The study involves an *in silico* biomodeling of the Cag protein of *H.pylori* responsible for gastroduodenal cancer as there is no three dimensional structure of the protein is available. The interaction of selected drugs and some plant alkaloids with the modeled receptor was done by rigid body docking techniques. CagA Screening of the functional inhibitors against this novel target may result in discovery of novel therapeutic compounds that can be effective against cancer.

## MATERIALS AND METHODS

### Retrieval of query sequence of *Helicobacter pylori* CagA and detection of best homologous templates

The protein sequence of CagA was retrieved from Swissprot database [10] (Uniprot ID: Q8RRV6) and the similarity searching was performed to detect the best homologues by P-BLAST and PSI-BLAST [11]. The sequences of CagA consist of 299 amino acids. Blast search among the known 3D structure revealed that CagA showing 31% sequence identity with the protein

Dihydroorotate Dehydrogenase (PDB ID 2B4G, chain A, resolution: 1.95 Å) of *Trypanosome brucii* and which was selected as suitable template

**Homology modeling**

The comparative homology modeling of Cag A protein was performed with Modeller 9v7 [12], computer program that models three-dimensional structures of proteins and their assemblies by satisfaction of spatial restraints. The required input files were prepared and it has run in phyton script. The modelled protein was visualized by PyMoL [13] and STRIDE [14], which uses hydrogen bond energy and main chain dihedral angles to recognize helix, coils and strands, was used to predict the secondary structure of the modeled Cag A protein. The target structure is then threaded with template to calculate RMSD by DaliLite tool [15]

**Refinement and validation of modeled Cag A**

The modeled CagA protein is further validated by various molecular dynamics and mechanics with the help of various force fields such as ANNOLEA [16], GROMOS [17] and VERIFY3D [18].The parameters included the covalent bond distances and angles, steriochemical validation and atom nomenclature were validated using PROCHECK [19]. The statistics of non-bonded interactions between different atom types was detected and value of the error function was analyzed by ERRAT [20].

**Molecular docking of selected ligand with modeled protein**

The chemical structure of CagA agonist and antagonists were extracted from NCBI PubChem [21] and KEGG databases [22]. Structures of 16 antibiotics and 09 plant alkaloids were selected based on the literature studies [23-25]. A rigid body docking was performed by HEX 6.1[26] by SP Fourier Transform, FFT steric Scan, FFT final Search and MM refinement. The clustering histogram with the scoring function was generated to analyze the binding energy of each selected conformations. The docked complex is viewed and the interaction residues of amino acids with the ligands were analyzed by PyMOL.

## RESULTS AND DISCUSSION

**Comparative protein structure modeling**

The sequence information of *Helicobacter pylori* CagA was described in the materials and methods. The pair wise alignment between target and template was performed(Fig.2).Secondary structure assignment by STRIDE provided physical skeleton of modeled proteins such as helices, extended strand and coil, our modeled protein primarily consists of alpha helices and random coil than extended strand. The 3D generated model was displayed by PyMol for visual interpretation( Fig.3)The superimposition with DaliLite was performed to analyses the backbone threading of template and target.There are 294 residues were aligned between the target and template and the Z score is 46.3.The root mean square

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deviation of C-alpha atoms are identified to be 1.2 A$^{o}$ which is significant and modeled protein is of good quality.

```
CAGA|Q8RRV6|      --ALADLKSLSFDLGKISDLQKSVKNVVIGTLVGNGLSKPEATTLTKNFSDIRKELNG-- 56
2B4G_A            GPGSMSLKVNILGHEFSNPFMNAAGVLCTTEEDLRRMTESESGSLIGKSCTLAPRTGNPE 60
                    .  .**   :.    . : ::.  :       . :::.*: :*  : . :  . ..

CAGA|Q8RRV6|      -KFFGN--SNNNNNGLKNNTEPIYAQVNKKTGQATSPEEPIYAQVARKVSAKIAQLNED 113
2B4G_A            PRYFGLPLGSINSMGLPNLGVDFYLSYAAQTHDYSRKPLFLSMSGLSVEESVEMVKKLVP 120
                   ::**   .. *. ** *    :* .   :. . : .*     : :: : *.::.:

CAGA|Q8RRV6|      TSAIIGKIDRFNKIASAVKG---VGVFSGAGRS--ANLEPIY-AQVARKVSAKIDQLNEA 167
2B4G_A            ITKEKGTILELNLSCPNVPGKPQVGYDFDTTRTYLQKVSEAYGLPFGVKMPPYFDIAHFD 180
                   :   *.* .:*  .. * *   **   .: *:   ::.  *   .. *:.. :*  :

CAGA|Q8RRV6|      TSAINRKIGQINKIASAGKGVGGFSGAGRSANPEPIYATIDFGEANQAGFPLRISAAVND 227
2B4G_A            MAAAVLNDFPLVKFITCVNSIGNGLVIDPANETVVIKPKQGFGGLGGKYVLPTALANVNA 240
                   :*   :   : *: :. :.:*.    . : :.  * .. .**  .   .     * **

CAGA|Q8RRV6|      LS-----KVGLSREQELTRRIGDLNQAVSEAKTGHFGNLEQKIDELKDFTKKNALKLLAE 282
2B4G_A            FFRRCPDKLVFGCGGVYSGEEAFLHILAGASMVQVGTALHDEGPIIFARLNKELQEIMTN 300
                  :      *: :.     : . . *:  .. : .     *.::   :    :*:  :::::

CAGA|Q8RRV6|      SAKQVPTSLQAKLDNYA 299
2B4G_A            KGYKTLDEFRGRVKTMD 317
                  .. :.  .::.::..
```

**Fig.2: Alignment between target (CagA) andTemplate (2B4G, ChainA)**

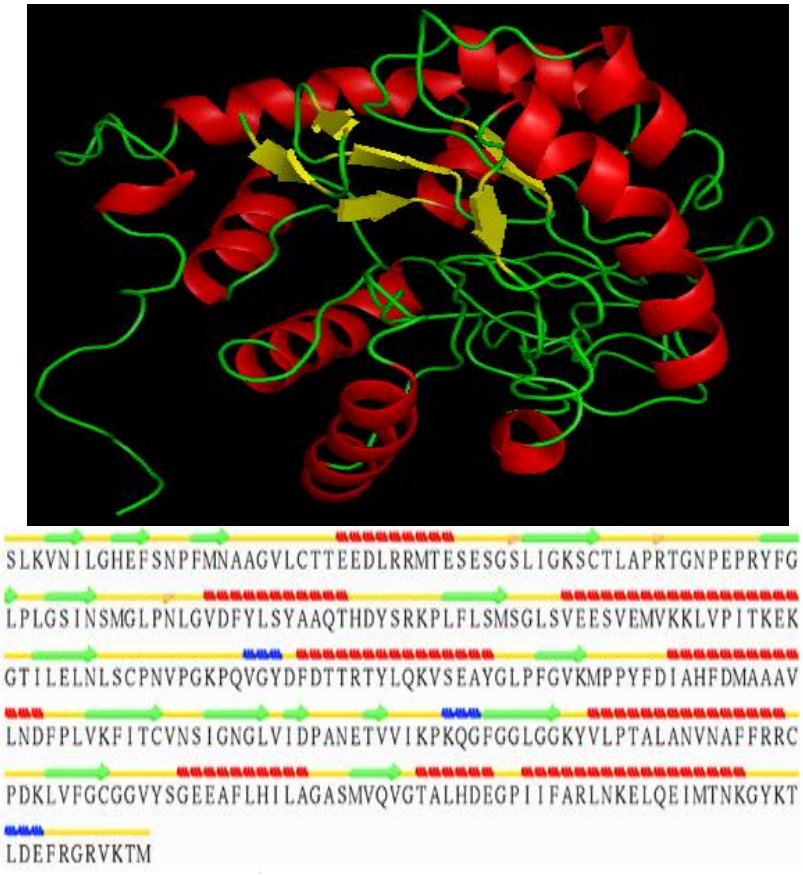


**Fig.3: 3 D model of CagA and its Predicted secondary structure**

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**Refinement and validation of Modeled structure**

The 3D structure model of CagA is validated by various empirical force fields of molecular dynamics. The steriochemical quality of modeled CagA was estimated by PROCHECK. The psi/phi angles of 89.5% residues included in the most favored regions, 10.5 residues lied in the additional allowed region and no residues fell in the generously allowed region and disallowed region of Ramchandran plot. Out of 311 residues 258 residues are constitutes non glycine and non proline residue, 31 residues for glycine and 20 residues for proline were observed in the steric counter diagram (Fig.4).Further refinement was done by other empirical force field such as ANOLEA, GROMOS and VERIFY 3D. All the refinement processes have given minimum energy levels of almost all the residues present in modeled protein. ERRAT computed the overall quality factor of model and which is identified to be 93.06, indicated the error values of individual residue are negligible (Fig.5). Thus statistical analysis suggests that the backbone conformation of our predicted model CagA was almost as good as that of X-ray Crystallographic structure of the template. The weighed RMSD of $C^{\alpha}$ trace between the template (2B4G) and the refined model of CagA was 1.2 $A^{o}$ with a significant Z-Score of 42.6.

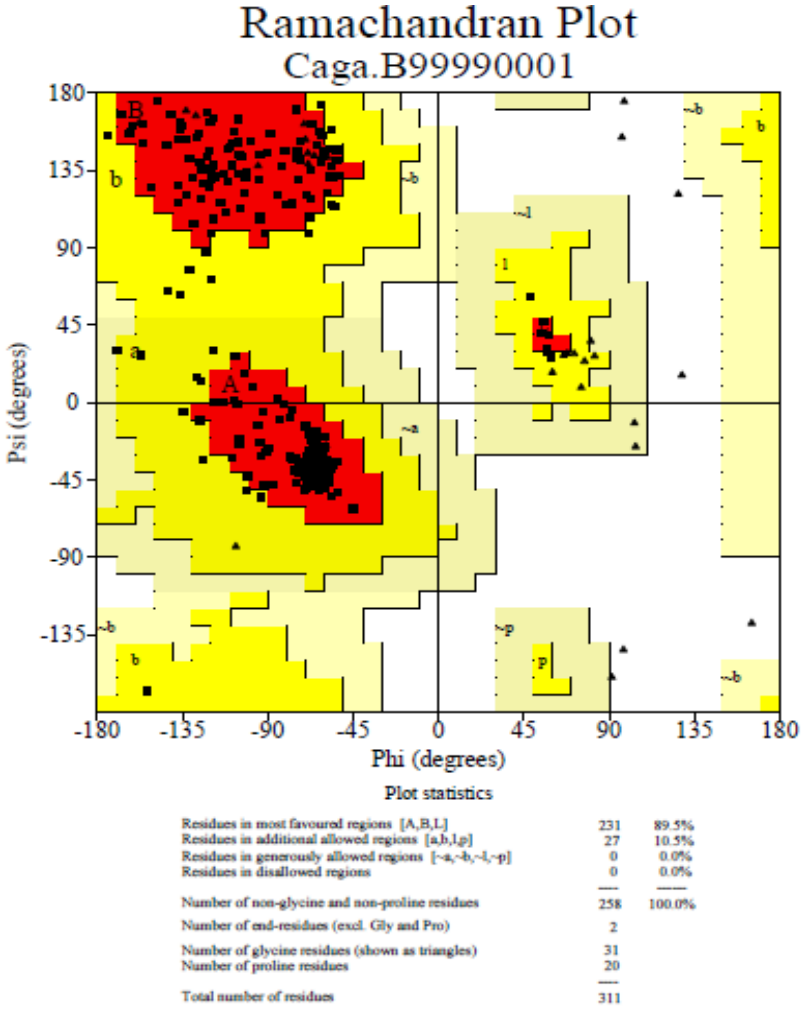


**Fig.4: Ramchandran plot of Modeled protein generated by PROCHECK**

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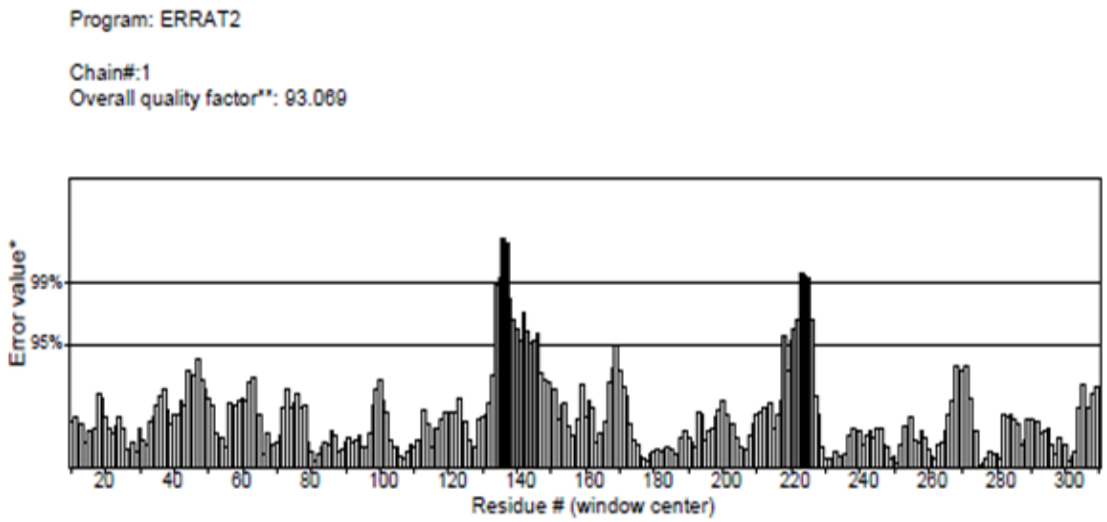


**Fig.5: Validation of modeled protein by ERRAT**

## Docking studies of modeled protein with best ligands

One of the excellent methods to design and optimize the drug against any molecule is through docking studies with selected ligands. So once a theoretical model of the Cag A was obtained its active site was predicted and characterized by docking with pharmacologically confirmed ligands with receptor. Sixteen antibiotics were docked with the best conformations of modeled protein and the binding energy was calculated.(Table.1)

**Table: 1. Docking binding energies of the different inhibitors (drug) against modeled CagA**

| S.No.. | NCBI PubChem ID | KEGG Drug/Compound ID | Name of the drug | Binding energy |
|---|---|---|---|---|
| 1. | CID 33613 | C06827 | AMOXICILLIN | -139.33 |
| 2 | CID 2756 | D00295 | CIMETIDINE | -135.70 |
| 3 | CID 84029 | D00276 | CLARITHROMYCIN | -146.27 |
| 4 | CID 3385 | D00584 | FLUOROURACIL | -139.89 |
| 5 | CID 60838 | D08086 | IRINOTECAN | -165.72 |
| 6 | CID 4173 | D00409 | METRONIDAZOLE | -83.47 |
| 7 | CID 5291 | D01441 | IMATINIB | -124.20 |
| 8 | CID 41867 | C11230 | EPIRUBICIN | -161.73 |
| 9 | CID 5353990 | D00201 | TETRACYCLIN | -132.96 |
| 10 | CID 3001055 | D00422 | RANITIDINE | -82.60 |
| 11 | CID 3325 | D00318 | FAMOTIDINE | -54.39 |
| 12 | CID 4594 | D00455 | OMEPRAZOLE | -66.25 |
| 13 | CID 31703 | D03899 | ADRIAMYCIN | -111.61 |
| 14 | CID CID 5746 | D0020 | MITOMYCIN | -122.84 |
| 15 | CID 498142 | D01363 | CARBOPLATIN | -113.42 |
| 16 | CID 9887054 | D01790 | OXALIPLATIN | -99.69 |

The clustering histogram shows that the antibiotics Clarithromycin, Amoxicillin, Andriamycin, Irinotecan and Epirubicin are the best inhibitors against CagA as the binding energy is minimum than other antibiotics. As per the literature studies it has been noticed that certain plant alkaloids are also have high inhibitory activity against Gastroduodenal cancer. We have selected nine plant alkaloids and the inhibitory activity was tested by docking (Table.2).

**Table:2. Binding energies of different plant alkaloids against modeled CagA**

| S.No. | NCBI PuChem ID | KEGG Drug/Compound ID | Name of the alkaloid | Binding energy |
|---|---|---|---|---|
| 1. | CID 2353 | D00092 | BERBERINE | -154.86 |
| 2. | CID 36314 | D00491 | TAXOL | -273.57 |
| 3. | CID 148124 | C11231 | TAXOTERE | -285.46 |
| 4. | CID 2554 | D00252 | FILDESIN | -170.36 |
| 5. | CID 60780 | D08680 | NAVELBINE | -305.70 |
| 6. | CID 5978 | C07204 | ONCOVIN | -168.83 |
| 7. | CID 13342 | C07201 | VINBLASTIN | -216.08 |
| 8. | CID 36462 | D00125 | ETOPOSIDE | -163.34 |
| 9. | CID 34698 | D02698 | TENIPOSIDE | -161.18 |

It has been revealed that Navelbine, a plant alkaloid extracted from the rosy periwinkle, *Catharanthus roseus* is the best inhibitor. Some other plant alkaloids such as Taxol, Docetaxel (Taxotere) and Vinblastin are also best inhibitors as docking gives minimum energy complex. The interaction of selected plant alkaloids with the modeled protein is given below.

**Interaction with Navelbine (Vinorelbin)**

Novelbine is the best inhibitor to be identified in our study. The clustering histogram of docked complex given a minimum energy score of -305.7 (Table.3) which shows better binding than other tested plant alkaloids. The important residues interacting with Navelbine are PHE 124, GLY133, ARG155 and GLY185 which forms strong hydrogen bond with the receptor (Fig.6)

**Interaction with Taxol and Taxotere**

The binding energy of taxol and taxotere with the modeled protein were identified as -273.57 and -285.46 which are far better than that of the binding of antibiotics. The main residue of modeled protein interacting with taxol is LEU291 (Fig.8)and taxotere is GLN206 (Fig.9)

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**Table.3: Clustering histogram of Docked complex (CagA and Navelbine) - correlation summary by RMS deviation and steric clashes**

| Clst | Soln | Models | Etotal | Eshape | Eforce | Eair | Vshape | Vclash | Bmp | RMS |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 000:000 | -305.7 | -305.7 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 2 | 000:000 | -296.2 | -296.2 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 6 | 000:000 | -288.8 | -288.8 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 8 | 000:000 | -285.5 | -285.5 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 9 | 000:000 | -285.0 | -285.0 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 19 | 000:000 | -275.4 | -275.4 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 23 | 000:000 | -274.0 | -274.0 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 44 | 000:000 | -266.1 | -266.1 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 47 | 000:000 | -265.9 | -265.9 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 55 | 000:000 | -264.7 | -264.7 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 61 | 000:000 | -264.2 | -264.2 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 62 | 000:000 | -264.1 | -264.1 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 67 | 000:000 | -263.5 | -263.5 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 73 | 000:000 | -262.4 | -262.4 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 97 | 000:000 | -259.9 | -259.9 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 104 | 000:000 | -258.8 | -258.8 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 144 | 000:000 | -254.8 | -254.8 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 157 | 000:000 | -253.6 | -253.6 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 175 | 000:000 | -252.6 | -252.6 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 1 | 188 | 000:000 | -251.6 | -251.6 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 3 | 000:000 | -292.6 | -292.6 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 4 | 000:000 | -289.1 | -289.1 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 10 | 000:000 | -283.5 | -283.5 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 18 | 000:000 | -275.6 | -275.6 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 21 | 000:000 | -274.3 | -274.3 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 26 | 000:000 | -272.6 | -272.6 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 37 | 000:000 | -267.9 | -267.9 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 45 | 000:000 | -266.1 | -266.1 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 50 | 000:000 | -265.7 | -265.7 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 56 | 000:000 | -264.7 | -264.7 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 60 | 000:000 | -264.2 | -264.2 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 70 | 000:000 | -263.1 | -263.1 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 71 | 000:000 | -262.9 | -262.9 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 91 | 000:000 | -260.4 | -260.4 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 102 | 000:000 | -259.0 | -259.0 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |
| 2 | 114 | 000:000 | -257.8 | -257.8 | 0.0 | 0.0 | 0.0 | 0.0 | -1 | -1.00 |

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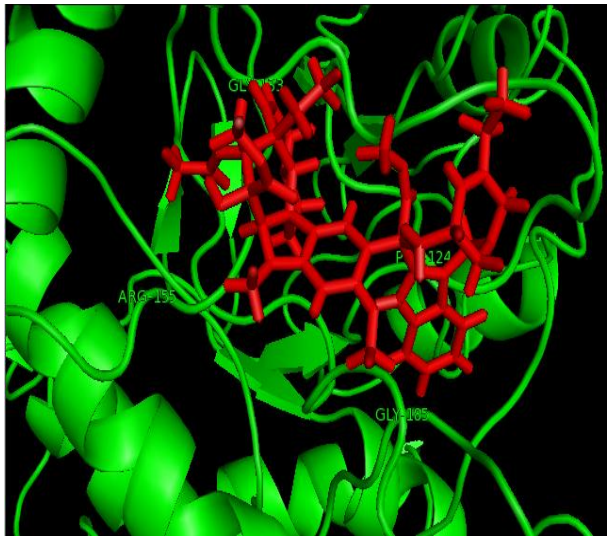


Fig.6: Modeled CagA is docked with Navelbine.Interacting Residues PHE124,GLY 133, ARG 155 and GLY18

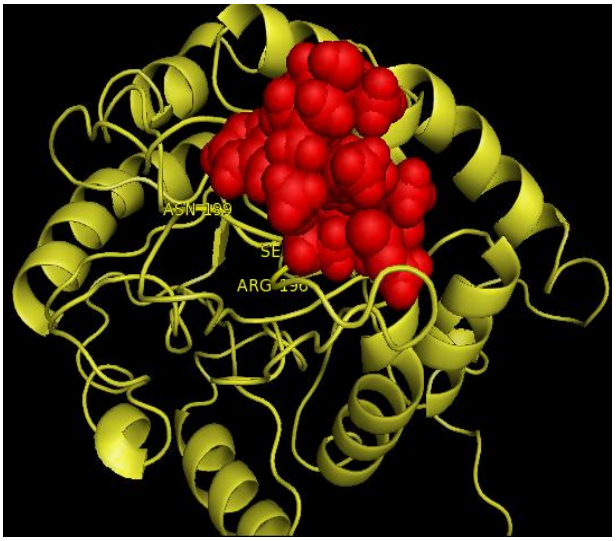


Fig.7 : Interaction of modeled protein with Vinblastin, Interacting residues are ARG196, SER197 and ASN199

## Interaction with Vinblastin

The clustering histogram of docking with vinblastin given a minimum binding energy of 216.08. The main residues interacting with vinblastin are ARG196, SER197 and ASN199 which form three bonds with the modeled protein (Fig.7)

The docking studies clearly shows that plant alkaloids have more inhibitory action against CagA protein than conventional antibiotics and Navelbine could be used as drug of choice against *Helicobacter pylori* gastroduodenal cancer.

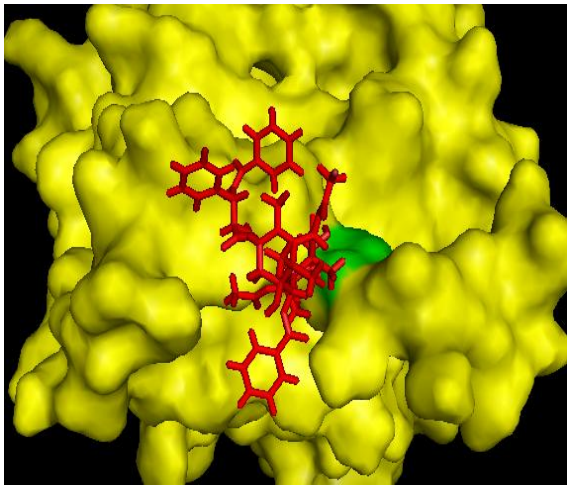

Fig.8 : intercation with taxol(interacting residue-LEU291

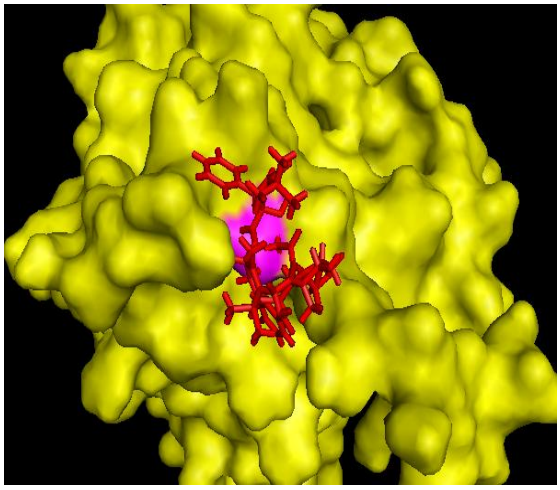

Fig.9: intercation with Taxotere(interacting residue-GLN206)

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## CONCLUSION

The 3D structure of Cag A was modeled based on the X-ray crystallographic structure of Dihydroorotase of *Trypanosoma brucii* (PDBID: 2B4G, chain A) taken as a template. The secondary structure of modeled protein consists of alpha helices and random coil than beta sheet. The RMSD value of the superimposed structure has estimated to be 1.2 $A^0$ and Z score is 46.3 which implies good quality of model. The model has further refined and validated by various molecular dynamics and mechanics tools. The PROCHECK Steriochemical validation of model shows 89. 5% and all the other empirical force fields have given satisfactory results. The interaction of various ligands to the modeled protein has studied by molecular docking. The results revealed that plant alkaloids like Navelbine, Docetaxel, Taxol and Vinblastin have better inhibitory action against CagA and given significant RMSD histogram compared to traditionally used antibiotics. Thus it could be concluded that purified form of above mentioned plant alkaloids, especially Navelbine could be used as suitable drug of choice for the eradication of gastroduodenal cancer.

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