# MOLECULAR DOCKING ANALYSIS OF MARINE BIOACTIVE COMPOUNDS TARGETING VACA AND CAGA VIRULENCE FACTORS OF HELICOBACTER PYLORI.

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## ABSTRACT

Helicobacter pylori (H. pylori), a Group I carcinogen, is associated with a range of gastrointestinal and extra-gastric diseases. This study aimed to evaluate the interaction of selected bioactive compounds, rosmarinic acid and stigmasterol, with key H. pylori virulence proteins, CagA (PDB ID: 4DVY) and VacA (PDB ID: 2QV3), using molecular docking analysis. The three-dimensional structures of the target proteins were retrieved from the Protein Data Bank, and docking simulations were performed using AutoDockTools 1.5.7, followed by interaction analysis in PyMOL. Rosmarinic acid demonstrated a binding energy of −5.1 kcal/mol and an inhibitory constant (Ki) of 18.35 mM against CagA, with interactions involving residues such as LEU475 and LYS613, while stigmasterol showed a binding energy of −3.39 kcal/mol and Ki of 3.25 mM, interacting with residues including ARG399 and GLU406. In the case of VacA, stigmasterol exhibited a binding energy of −4.38 kcal/mol (Ki 6.16 mM), whereas rosmarinic acid showed −2.93 kcal/mol (Ki 7.1 mM), with interactions observed at residues such as LYS680 and TYR729. These findings indicate that both compounds are capable of interacting with key residues of CagA and VacA proteins, with variations in binding affinity; however, as molecular docking provides only predictive insights into ligand–protein interactions, further experimental validation is required to confirm their biological activity and potential therapeutic relevance.

**KEYWORDS:** H. pylori, oral infection, malignancy, head and neck cancer, oncoproteins, molecular docking.

## INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative, spiral-shaped bacterium that chronically colonizes the human gastric mucosa and infects approximately half of the global population, with higher prevalence reported in developing regions.[1] The organism possesses unique adaptive mechanisms, including urease production, which enables survival in the highly acidic gastric environment. Persistent infection with H. pylori is a well-established risk factor for several gastrointestinal disorders, including chronic gastritis, peptic ulcer disease, and gastric carcinoma.[2] The pathogenicity of H. pylori is largely attributed to its virulence factors, which facilitate colonization, immune evasion, and host tissue damage. Chronic colonization leads to sustained inflammatory responses that may induce epithelial alterations and increase the risk of malignant transformation.[3]

Emerging evidence suggests that H. pylori infection may also be associated with extra-gastric conditions, including diseases of the oral cavity and head and neck region.[4–6] Although the exact mechanisms remain unclear, chronic inflammation, immune modulation, and possible colonization of the oral environment have been proposed as contributing factors. Persistent inflammatory signaling is known to promote genomic instability, dysregulated cell proliferation, and impaired apoptosis, all of which are implicated in carcinogenesis.[7] Furthermore, the detection of H. pylori in oral biofilms and saliva supports the possibility of its involvement in local pathological processes.[5, 6] However, current evidence remains inconclusive, highlighting the need for further investigation into the molecular interactions underlying these associations.

Among the various virulence determinants of H. pylori, cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) are extensively studied due to their critical roles in host–pathogen interactions. CagA is translocated into host cells, where it disrupts intracellular signaling pathways, alters cytoskeletal organization, and promotes inflammatory responses.[8, 9] Similarly, VacA contributes to cellular damage by inducing vacuolation, mitochondrial dysfunction, and modulation of immune responses, thereby facilitating bacterial persistence.[10] These proteins are therefore considered important molecular targets for investigating potential inhibitory compounds.

Natural bioactive compounds have gained considerable attention as potential modulators of microbial virulence due to their diverse pharmacological properties. Rosmarinic acid, a polyphenolic compound, has demonstrated antioxidant, anti-inflammatory, and antimicrobial activities in several experimental models.[11,12] Stigmasterol, a plant-derived phytosterol, has also been reported to exhibit anti-inflammatory and cytomodulatory effects.[13,14] While these compounds have shown biological activity in various contexts, their direct interaction with H. pylori virulence proteins such as CagA and VacA has not been extensively explored. Investigating such interactions using computational approaches may provide preliminary insights into their potential inhibitory behavior.

Molecular docking is a widely used in silico technique that predicts the binding affinity and interaction patterns between ligands and target proteins, thereby aiding in the identification of potential lead compounds.[15] In this context, the present study aims to evaluate the interaction of rosmarinic acid and stigmasterol with the H. pylori virulence proteins CagA and VacA using molecular docking analysis. This approach seeks to provide a computational basis for understanding ligand–protein interactions, which may guide future experimental studies.

### Material and Methodology

The three-dimensional crystal structures of the Helicobacter pylori virulence proteins CagA (PDB ID: 4DVY) and VacA (PDB ID: 2QV3) were retrieved from the Protein Data Bank (PDB).[16] Protein preparation was performed using Molegro Molecular Viewer version 7.0, where co-crystallized ligands, water molecules, and non-essential chains were removed to obtain a clean protein structure suitable for docking. Polar hydrogen atoms were added, and Kollman charges were assigned using

AutoDockTools version 1.5.7 to ensure accurate representation of electrostatic interactions.[17]

The ligand structures of rosmarinic acid and stigmasterol were obtained from the PubChem database (CID: 5281792 and 5280794, respectively) in SDF format and converted to PDB format using Open Babel.[18] Ligand preparation was carried out using AutoDockTools 1.5.7, where torsional degrees of freedom were defined (1–6 rotatable bonds), hydrogen atoms were added, Gasteiger charges were assigned, and aromaticity was detected. These steps are essential for enabling flexible ligand docking and accurate conformational sampling.[19]

The active site regions of CagA and VacA were identified based on previously reported binding residues and structural analysis of the proteins.[10,16] A grid box was generated using AutoGrid with dimensions of 50 × 64 × 78 Å and a grid spacing of 0.5 Å, centered at coordinates x = 83.35, y = 49.60, and z = 50.60, encompassing the predicted binding pockets.[17]

Molecular docking simulations were performed using AutoDock 4.2, employing the Lamarckian Genetic Algorithm (LGA), which is widely used for predicting ligand–protein interactions.[20] For each ligand–protein complex, 100 independent docking runs were carried out with a population size of 250, a mutation rate of 0.02, and a crossover rate of 0.8. The maximum number of energy evaluations was set to $2.5 \times 10^6$ to ensure adequate conformational sampling. Docking parameter files (DPF) were generated for each simulation.

To validate the docking protocol, standard docking procedures were followed, and the consistency of binding poses was assessed through clustering analysis using a root mean square deviation (RMSD) tolerance of 1.0 Å.[21] The lowest energy conformation from the most populated cluster was selected as the representative binding mode. Binding energy values (kcal/mol) and estimated inhibition constants (Ki) were recorded as indicators of interaction strength. The total energy represents the combined contribution of intermolecular interaction energy and torsional free energy.

The resulting protein–ligand complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer version 25.1.0, enabling identification of key interacting residues and types of interactions such as hydrogen bonding, hydrophobic interactions, and electrostatic contacts.[22]

## RESULTS

### Molecular Docking Analysis of CagA Protein

The docking results of rosmarinic acid and stigmasterol with the CagA oncoprotein of Helicobacter pylori are summarized in Table 3. Rosmarinic acid exhibited a binding energy of −5.1 kcal/mol, with a ligand efficiency of −0.2 kcal/mol and an estimated inhibition constant (Ki) of 18.35 mM. The intermolecular energy was calculated as −8.68 kcal/mol, and the total energy was −3.7 kcal/mol. The ligand interacted with residues LEU475, ASP477, LYS613, LYS617, GLU620, and ARG624 in chain A (Figure 1).

Stigmasterol demonstrated a binding energy of −3.39 kcal/mol, with a ligand efficiency of −0.08 kcal/mol and a Ki value of 3.25 mM. The intermolecular energy was −6.97 kcal/mol, and the total energy was −3.61 kcal/mol. The interacting residues identified for stigmasterol included ARG399, GLU406, GLN410, LYS421, LYS425, ALA601, and LYS604 in chain A (Figure 1).

### Molecular Docking Analysis of VacA Protein

The docking results of rosmarinic acid and stigmasterol with the VacA protein are presented in Table 4. Rosmarinic acid showed a binding energy of −2.93 kcal/mol, with a ligand efficiency of −0.11 kcal/mol and a Ki value of 7.1 mM. The intermolecular energy was −6.51 kcal/mol, and the total energy was −3.35 kcal/mol. The interacting residues included ALA431, GLY432, SER433, PHE461, PHE483, ASN484, SER504, THR505, ASN506, GLY527, GLU528, and TYR529 in chain A (Figure 2).

Stigmasterol exhibited a binding energy of −4.38 kcal/mol, with a ligand efficiency of −0.11 kcal/mol and a Ki value of 6.16 mM. The intermolecular energy was −7.96 kcal/mol, and the total energy was −3.73 kcal/mol. The residues involved in interaction were LYS680, TYR729, ASN732, ARG734, CYS738, VAL739, ALA748, and CYS749 in chain A (Figure 2).

## DISCUSSION

Helicobacter pylori (H. pylori) infection is widely recognized for its role in gastrointestinal diseases, with its pathogenicity primarily mediated by virulence factors such as cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA).[8–10] These proteins contribute to host cell dysfunction through disruption of intracellular signaling pathways, induction of cellular stress, and modulation of immune responses. Due to their critical involvement in bacterial pathogenic mechanisms, they are considered important molecular targets for computational and experimental investigations.

In the present study, molecular docking analysis was performed to evaluate the interaction of rosmarinic acid and stigmasterol with CagA and VacA proteins. The docking results demonstrated that both compounds were able to bind within the predicted active regions of these proteins, with variations observed in binding energy values and interacting residues. Rosmarinic acid exhibited comparatively lower binding energy toward CagA, whereas stigmasterol showed relatively favorable interaction with VacA. These differences may reflect variations in molecular structure, polarity, and conformational flexibility of the ligands, which influence their binding orientation and interaction profiles.

It is important to note that molecular docking provides a theoretical prediction of ligand–protein interactions based on structural complementarity and energy minimization.[15] Therefore, the binding affinities reported in this study should be interpreted as indicative of potential interactions rather than definitive evidence of biological activity. The interaction of ligands with specific amino acid residues identified in this study may provide a basis for further structural and functional investigations.

Previous studies have reported that rosmarinic acid possesses antimicrobial, antioxidant, and anti-inflammatory properties, and has demonstrated biological activity in various in vitro models.[11,12] Similarly, stigmasterol has been shown to exhibit anti-inflammatory and cytomodulatory effects in experimental studies.[13,14] While these findings support the potential biological relevance of these compounds, their direct inhibitory effects on H. pylori virulence proteins such as CagA and VacA have not been extensively validated experimentally.

The present findings therefore provide preliminary computational insight into the interaction of these natural compounds with key H. pylori virulence factors. However, several limitations should be considered. Molecular docking does not account for dynamic protein behavior, solvent effects, or cellular complexity, which may influence actual biological interactions. Additionally, the absence of experimental validation limits the ability to confirm inhibitory activity or therapeutic relevance.

Future studies involving molecular dynamics simulations,

in vitro assays, and in vivo models are required to further investigate the stability, specificity, and biological effects of these interactions. Such approaches would provide a more comprehensive understanding of the potential role of these compounds in modulating H. pylori pathogenic mechanisms.

## CONCLUSION

This study employed molecular docking analysis to evaluate the interaction of rosmarinic acid and stigmasterol with key Helicobacter pylori virulence proteins, CagA and VacA. The findings indicate that both compounds exhibit binding affinity toward these target proteins, with variations observed in interaction profiles and energy values. These results provide preliminary computational insight into ligand–protein interactions involving important virulence factors of H. pylori. However, as molecular docking represents a predictive approach, the biological activity and inhibitory potential of these compounds cannot be confirmed without further investigation. Additional studies, including molecular dynamics simulations and experimental validation through in vitro and in vivo models, are necessary to determine their functional relevance and potential applicability.

## REFERENCES


1. Kazanowska-Dygdała M, Duś I, Radwan-Oczko M. The presence of Helicobacter pylori in oral cavities of patients with leukoplakia and oral lichen planus. J Appl Oral Sci. 2016;24(1):18–23.

2. Wang L, Tay ACY, Marshall BJ. Towards the eradication of Helicobacter pylori infection: rapid diagnosis and precision treatment. BoD – Books on Demand; 2024.

3. Bhattacharjee A, Sahoo OS, Sarkar A, Bhattacharya S, Chowdhury R, Kar S, Mukherjee O. Infiltration to infection: key virulence players of Helicobacter pylori pathogenicity. Infection. 2024;52(2):345-84.

4. Kannan N, Pandiar D, Subramanian R, Krishnan RP, S C. Helicobacter pylori-positive oral squamous cell carcinoma demonstrates higher pathological tumor staging and poorer overall survival. J Stomatol Oral Maxillofac Surg. 2024;101952.

5. Adler I, Muiño A, Aguas S, Harada L, Diaz M, Lence A, et al. Helicobacter pylori and oral pathology: relationship with gastric infection. World J Gastroenterol. 2014;20(29):9922–35.

6. Kayali S, Manfredi M, Gaiani F, Bianchi L, Bizzarri B, Leandro G, et al. Helicobacter pylori, transmission routes and recurrence of infection: state of the art. Acta Biomed. 2018;89(8 Suppl):72–76.

7. Zahir SO, El Khadir M, Boukhris SA, Benajah D-A, Ibrahimi SA, Chbani L, et al. Helicobacter pylori vacA allelic combination, dupA, cagE and cagA genotypes and their associations with gastric diseases among Moroccan population. Jpn J Infect Dis. 2024.

8. Hatakeyama M. Impact of the Helicobacter pylori oncoprotein CagA in gastric carcinogenesis. Curr Top Microbiol Immunol. 2023;444:239–57.

9. Takahashi-Kanemitsu A, Lu M, Knight CT, Yamamoto T, Hayashi T, Mii Y, et al. The CagA oncoprotein disrupts Wnt/PCP signaling and promotes hyperproliferation of pyloric gland base cells. Sci Signal. 2023;16(794):eabp9020.

10. Vieira RV, Peiter GC, de Melo FF, Zarpelon-Schutz AC, Teixeira KN. Prospective analysis of medicinal plant activity on the CagA oncoprotein from Helicobacter pylori. World J Clin Oncol. 2024;15(5):653–63.

11. Priyadarshini G, Gheena S, Dilipan E, Ramani P. Targeting oral cancer: in silico docking studies of phytochemicals on oncogenic molecular markers. Asian Pac J Cancer Prev. 2024;25(6):2069–75.

12. Ijaz S, Iqbal J, Abbasi BA, Ullah Z, Yaseen T, Kanwal S, et al. Rosmarinic acid and its derivatives: current insights on anticancer potential and other biomedical applications. Biomed Pharmacother. 2023;162: 114687.

13. Zhao J, Xu L, Jin D, Xin Y, Tian L, Wang T, et al. Rosmarinic acid and related dietary supplements: potential applications in the prevention and treatment of cancer. Biomolecules. 2022;12(10).

14. Luo Y, Ma Z, Xu X, Qi H, Cheng Z, Chen L. Anticancer effects of rosmarinic acid in human oral cancer cells mediated via endoplasmic reticulum stress, apoptosis, G2/M cell cycle arrest and inhibition of migration. J BUON. 2020;25(2):1245–50.

15. Radziejewska I, Supruniuk K, Bielawska A. Anti-cancer effect of combined action of anti-MUC1 and rosmarinic acid in gastric cancer cells. Eur J Pharmacol. 2021;902:174119.

16. Vieira RV, Peiter GC, de Melo FF, Zarpelon-Schutz AC, Teixeira KN. In silico analysis of medicinal plants targeting the CagA oncoprotein of Helicobacter pylori. World J Clin Oncol. 2024;15(5):653–63.

17. Hannan MA, Sohag AAM, Dash R, Haque MN, Mohibbullah M, Oktaviani DF, et al. Phytosterols of marine algae: insights into potential health benefits and molecular pharmacology. Phytomedicine. 2020;69:153201.

18. Bakrim S, Benkhaira N, Bourais I, Benali T, Lee L-H, El Omari N, et al. Health benefits and pharmacological properties of stigmasterol. Antioxidants (Basel). 2022;11(10).

19. Fong P, Hao C-H, Io C-C, Sin P-I, Meng L-R. In silico and in vitro effects of combinations of phytochemicals and antibiotics. Molecules. 2019;24(19).

20. Bae H, Song G, Lim W. Stigmasterol induces ovarian cancer cell apoptosis via endoplasmic reticulum and mitochondrial dysfunction. Pharmaceutics. 2020;12(6).

21. Begum NF, Ramani P, Doble M, Ramasubramanian A. Identification of potential lead compounds against BCL-2 through in silico screening of phytochemicals for oral squamous cell carcinoma management. Curr Cancer Ther Rev. 2024;20(4):410–16.

22. Begum NF, Ramadoss R, Yadalam PK, Ramani P, Ramalingam K. Phytochemical targeting of nerve growth factor by thymoquinone and cuscutin: a molecular dynamics simulation study. Cureus. 2024;16(7):e63727.

23. Kannan N, Gheena S, Ramani P, Shanmugam R, Ramalingam K. A novel gum paint formulation derivefrom licorice and triphala: characteristics and clinical significance for improved oral health. Cureus. 2024.

24. Dharini S, Ramani P, Doble M, Ramasubramanian A. Ferroptosis-mediated novel drug design approach in oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2023;24(7):2321–27.

**Table 1 Structure of Receptor Molecule retrieved from Protein Bata Bank.**

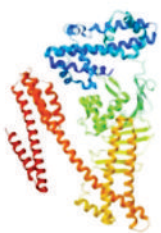
| SN | Structure Name | Structure | Structure ID | No. of Chains and Sequence Length |
|---|---|---|---|---|
| 1 | *Helicobacter pylori* CagA oncoprotein |  | PDB ID: 4DVY | A (876) |
| 2 | *Helicobacter pylori* (VacA) Vacuolating Toxin p55 Domain |  | PDB ID: 2QV3 | A (457) |

**Table 2 -Structure of Ligands retrieved from PubChem database.**

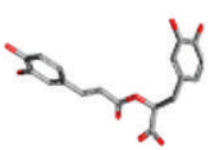
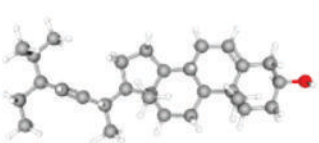
| SN. | Structure Name | Structure | Structure ID | Molecular Formula |
|---|---|---|---|---|
| 1 | Rosmarinic Acid |  | PubChem CID: 5281792 | $C_{18}H_{16}O_8$ |
| 2 | Stigmasterol |  | PubChem CID: 5280794 | $C_{29}H_{48}O$ |

**Table 3. Docking parameters of rosmarinic acid and stigmasterol with CagA protein (PDB ID: 4DVY).**

| RMSD | Rosmarinic Acid | Stigmasterol |
|---|---|---|
| Binding Energy | -5.1 kcal/mol | -3.39 kcal/mol |
| Ligand Efficiency | -0.2 kcal/mol | -0.08 kcal/mol |
| Inhibitory Constant | 18.35 mM | 3.25 mM |
| Intermolecular energy | -8.68 kcal/mol | -6.97 kcal/mol |
| Total Energy | -3.7 kcal/mol | -3.61 kcal/mol |
| Amino acid residue | Chain A: LEU475; ASP477; LYS613; LYS617; GLU620; ARG624; | Chain A: ARG399; GLU406; GLN410; LYS421; LYS425; ALA601; LYS604 |

Note: Binding energy is expressed in kcal/mol. Ki: inhibition constant. RMSD: root mean square deviation.

**Table 4. Docking parameters of rosmarinic acid and stigmasterol with VacA protein (PDB ID: 2QV3).**

| RMSD | Rosmarinic Acid | Stigmasterol |
|---|---|---|
| Binding Energy | -2.93 kcal/mol | -4.38 kcal/mol |
| Ligand Efficiency | -0.11 kcal/mol | -0.11 kcal/mol |
| Inhibitory Constant | 7.1 mM | 6.16 mM |
| Intermolecular energy | -6.51 kcal/mol | -7.96 kcal/mol |
| Total Energy | -3.35 kcal/mol | -3.73 kcal/mol |
| Amino acid residue | Chain A: ALA431; GLY432; SER433; PHE461; PHE483; ASN484; SER504; THR505; ASN506; GLY527; GLU528; TYR529 | Chain A: LYS680; TYR729; ASN732; ARG734; CYS738; VAL739; ALA748; CYS749 |

Note: Binding energy is expressed in kcal/mol. Ki: inhibition constant. RMSD: root mean square deviation.

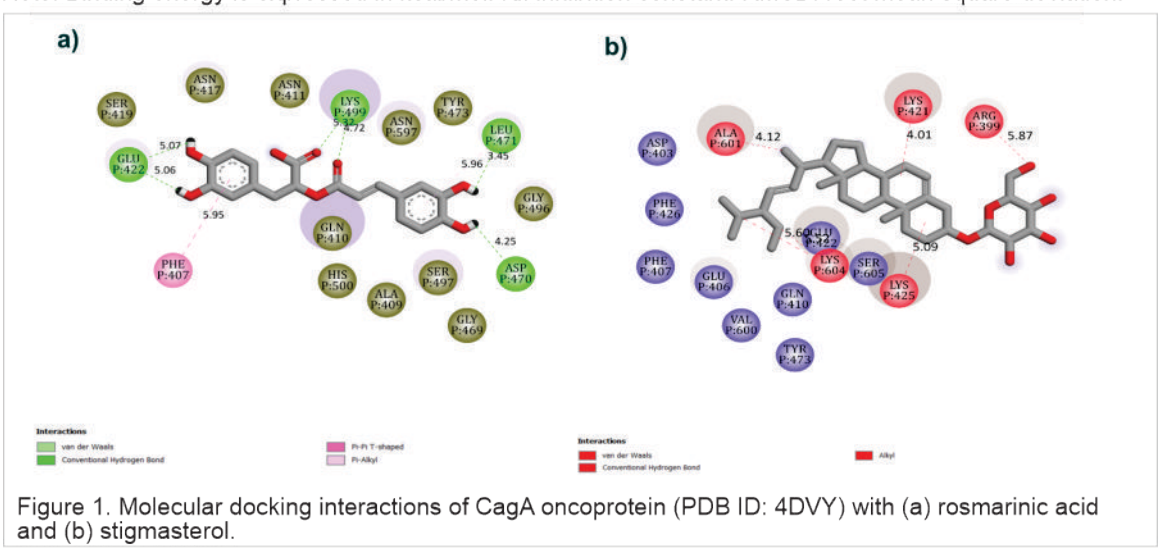



Figure 1. Molecular docking interactions of CagA oncoprotein (PDB ID: 4DVY) with (a) rosmarinic acid and (b) stigmasterol.

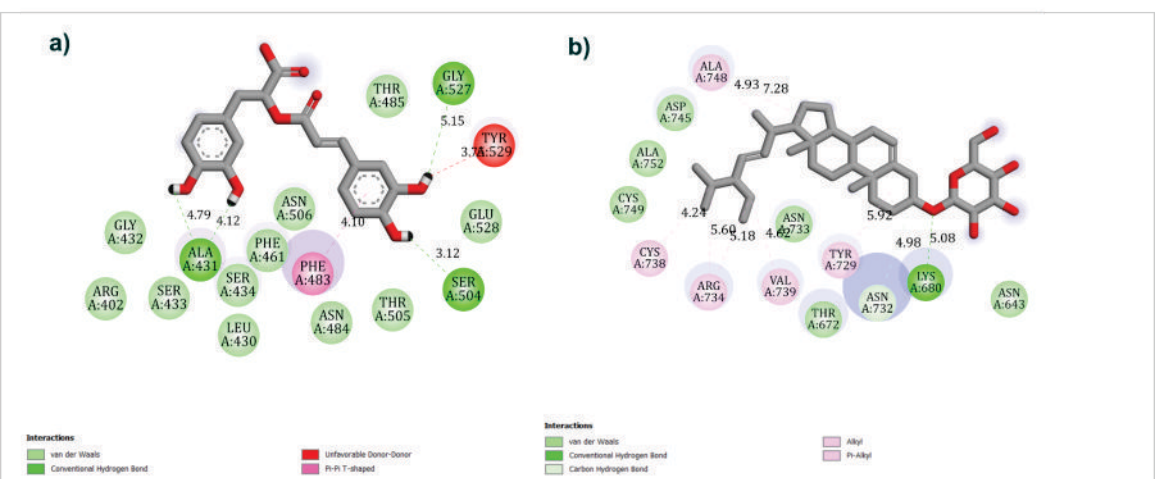



Figure 2. Molecular docking interactions of VacA toxin (PDB ID: 2QV3) with (a) rosmarinic acid and (b) stigmasterol..