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In Silico Screening of *Laminaria japonica* Ligands as Potential Inhibitors of DPP-4 for Type 2 Diabetes Treatment

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ABSTRACT

Type 2 diabetes mellitus (DM2) is an increasing global health issue marked by insulin resistance and altered glucose homeostasis. Dipeptidyl peptidase-4 (DPP4) is crucial in modulating glucose concentrations through inactivation of incretin hormones. Inhibition of DPP4 constitutes a therapeutic approach for the treatment of DM2. *Laminaria japonica*, a marine alga, possesses bioactive substances such as fucoidan, beta-ionone, and laminine, which exhibit potential anti-diabetic activities. The chemical interactions of these drugs with DPP4 are still inadequately understood. This work sought to examine the binding interactions of the main *Laminaria japonica* ligands with DPP4 by molecular docking and molecular dynamics simulations. The study aimed to forecast the binding affinity, stability, and critical interactions of these ligands inside the active site of DPP4. Molecular docking was performed to determine binding energies, succeeded by molecular dynamics simulations lasting 100 ns to assess complex stability. The top three ligands obtained from docking were fucoidan, beta-ionone, and laminin, with binding energies of -5.79 kcal/mol, -5.64 kcal/mol, and -5.6 kcal/mol, respectively, compared to the native ligand PF2, which had a binding energy of -9.21 kcal/mol. However, beta-ionone demonstrated the best ΔTOTAL value of -24.73 kcal/mol in the MD simulation. In conclusion, *in silico* research indicated that beta-ionone from *Laminaria japonica* was a potential candidate for DPP4 inhibition and may act as lead compounds for the formulation of innovative anti-diabetic drugs. Additional *in vitro* and *in vivo* investigations are necessary to corroborate these results.

Keywords: *Laminaria japonica*, Dipeptidyl peptidase-4 inhibition, Molecular docking, Type 2 diabetes, Ligand interaction

Introduction

Type 2 diabetes mellitus (DM2) is a chronic metabolic condition marked by insulin resistance and gradual deterioration of pancreatic beta cell function, resulting in increased blood glucose levels.[1] The worldwide incidence of DM2 has increased rapidly due to variables including sedentary behavior, obesity, and inadequate eating practices.[2] This disorder is linked to severe sequelae, such as cardiovascular disease, renal failure, and neuropathy, making it one of the most significant public health challenges worldwide.[3] Management of DM2 often encompasses lifestyle modifications and pharmaceutical therapies designed to improve insulin sensitivity or increase insulin production. However, these therapies often have restricted effectiveness and may induce harmful side effects. Consequently, there is an increasing demand for innovative therapeutic strategies that can proficiently regulate blood glucose levels with minimal adverse consequences. *Laminaria japonica*, a marine algae prevalent in East Asian waters, has garnered interest for its array of bioactive chemicals that may offer health advantages.[4]

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Laminaria japonica, traditionally used in Chinese and Japanese medicine, is recognized for its anti-inflammatory, antioxidant, and anti-obesity attributes.[5] Recent research has revealed multiple chemicals in *Laminaria japonica* with possible anti-diabetic properties, including fucoidan, laminine, and beta-ionone.[6] These substances have shown the potential to modulate glucose metabolism, block critical enzymes associated with carbohydrate digestion, and enhance insulin sensitivity. Due to the increasing interest in marine-derived natural products as therapeutic agents, *Laminaria japonica* presents a viable source of novel lead compounds for the treatment of DM2.[7] However, the precise methods by which these drugs interact with molecular targets associated with DM2, including the enzyme dipeptidyl peptidase-4 (DPP4), are mostly unexamined. DPP4 is a pivotal enzyme that facilitates the inactivation of incretin hormones, essential for glucose regulation.[8] Inhibition of DPP4 can increase the efficacy of incretins, therefore increasing insulin production and lowering blood glucose levels. Current DPP4 inhibitors, including sitagliptin and vildagliptin, have demonstrated clinical efficacy in the management of DM2.[9] However, prolonged use of these inhibitors is associated with possible adverse consequences, such as an increased risk of infections and pancreatic complications.[10] As a result, the exploration of natural DPP4 inhibitors of botanical and marine origin has emerged as a promising strategy to create safer and more effective therapies for DM2. In this context, *Laminaria japonica* serves as a significant source of bioactive chemicals that may function as DPP4 inhibitors; however, additional studies are required to validate their efficacy as therapeutic agents. Notwithstanding the recognized bioactive potential of *Laminaria japonica*, there remains a considerable research gap in elucidating the molecular interactions of its components with DPP4. Prior research has predominantly concentrated on *in vitro* and *in vivo* evaluations of the

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anti-diabetic properties of these drugs;[11] however, there is scant information regarding their molecular interactions with DPP4. Computational methods, such as molecular docking and molecular dynamics simulations,[12] can provide significant information on the binding affinity, stability, and interaction patterns of these ligands with DPP4, thus improving the knowledge of their potential as DPP4 inhibitors. This research seeks to fill this gap by examining the interaction of the principal ligands of *Laminaria japonica* with DPP4 using *in silico* techniques.

The main objective of this study was to analyze the binding interactions of specific *Laminaria japonica* ligands with DPP4 and evaluate their potential as lead compounds for the treatment of DM2. This research employed molecular docking and molecular dynamics simulations to predict the binding affinity and stability of ligands, including fucoidan, laminin, and beta-ionone, with the active site of DPP4. The work aimed to investigate critical chemical interactions, such as hydrogen bonding and *van der Waals* forces, that underpin the inhibitory efficacy of these ligands against DPP4. This research sought to find potential lead compounds for the development of natural DPP4 inhibitors for the control of DM2.

The methodology used molecular docking to determine the binding mechanism of the ligands at the active site of DPP4, followed by molecular dynamics simulations to assess the stability and flexibility of the ligand-DPP4 complexes over time. The docking approach was confirmed by redocking the native ligand (PF2) into the active site of DPP4 and assessing the root mean square deviation (RMSD) between the re-docked and crystallographic structures. Ligands exhibiting high binding affinity and advantageous interactions with DPP4 in docking studies were subsequently analyzed through molecular dynamics simulations to evaluate their stability in physiological circumstances. The simulations provided insight into the dynamic behavior of the ligand-DPP4 complexes, encompassing variations in RMSD, root mean square fluctuation (RMSF), and components of interaction energy.

This study sought to investigate the potential of *Laminaria japonica* bioactive chemicals as natural DPP4 inhibitors by *in silico* analysis. This research utilized molecular docking and molecular dynamics simulations to thoroughly assess the binding interactions and stability of these drugs with DPP4. The findings of this study improved existing knowledge on natural DPP4 inhibitors and provided significant information for the potential creation of new anti-diabetic treatments derived from natural marine materials.

Materials and Methods

Hardware

The molecular docking and molecular dynamics simulations were performed using an HP Z640 WorkStation Server with the following specifications: a Xeon E5 2690 V3 CPU, augmented by 32 GB of RAM and an RTX 3060 graphics card, which furnished the requisite computing capacity for the simulations.

Software

Several software tools were employed in the research to build the studying the molecular interactions based on molecular docking and molecular dynamics. AutoDock[13] and Gromacs 2023[14] were used for molecular docking and molecular dynamics, respectively. Avogadro[15] is a free and open-source application for editing and visualizing molecular structures. Discovery Studio Visualizer 2021[16], was used for viewing and studying the structures and characteristics of complexes.

Preparation of enzyme structure

The macromolecule linked to PDB ID 3F8S, which is integral to the DPP4 enzyme, was obtained from the Protein Data Bank at www.rcsb.org.[17] This phase supplied the structural data essential for examining the interactions between the macromolecule and various ligands. Subsequently, the downloaded structure was refined to separate solely the macromolecule and its native ligand, PF2. This entailed the elimination of superfluous molecules, water, and heteroatoms that were not immediately pertinent to the investigation. The purified structure, which comprises only the macromolecule and PF2, was subsequently saved in *.pdb format, facilitating additional molecular docking and analysis.

Preparation of ligands

Ligand data for *Laminaria japonica* compounds were acquired in the format *.3d.mol format from the Knapsack database at http://knapsack3d.sakura.ne.jp/.[18] This database provided preliminary structural data for the ligands utilized in this research.

The downloaded ligands were evaluated according to Lipinski's five rules to ascertain their druglikeness and probable bioavailability.[19] Only ligands that met all Lipinski criteria—molecular weight < 500 Da, octanol-water partition coefficient (LogP) < 5, hydrogen bond donors 5, and hydrogen bond acceptors 10—were chosen for subsequent investigation. This stage confirmed the appropriateness of the compounds for molecular docking research.The selected ligands underwent geometry tuning to enhance their structures. The MMFF94 force field was utilized within the Avogadro software for this procedure.[20] Geometry optimization rectified early structural aberrations and ensured that the ligands assumed their most stable conformations.

Following optimization, the ligands' structures were transformed into the *.pdb format utilizing Avogadro. This format is compatible with molecular docking applications, facilitating subsequent docking simulations and analyses. The conversion step guaranteed that the ligands were in an appropriate format for incorporation into the molecular docking method.

Molecular docking simulation

The docking validation process was performed to evaluate the interaction of *Laminaria japonica* ligands with DPP4 using AutoDock 4.2. This involved re-docking the native ligand, PF2, into the active site of the DPP4 structure designated by PDB ID 3F8S. The precision of the docking technique was assessed by juxtaposing the re-docked conformation of PF2 with its reference crystallographic structure, utilizing the root mean square deviation (RMSD) as the principal metric.[21,22] Ideally, the RMSD should be below 2 Å to validate the reliability of the docking technique.[23] The grid box specifications for this validation comprised npts configured at 50 × 50 × 50 with a spacing of 0.375 Å and a population size of 100 for the genetic algorithm.

Subsequently for validation, molecular docking simulations were conducted for the test ligands. The ligands were bound to the DPP4 macromolecular structure using the parameters of the grid box derived from the validation phase. This methodology guaranteed that the docking simulations were conducted using the approved settings, thereby ensuring consistency and dependability in the interaction predictions for the ligands examined.

Scoring and results interpretation

After the initial docking, AutoDock evaluated the binding postures that were generated by utilizing energy estimations. The binding poses with the lowest binding energy were determined to be the most advantageous. The binding poses with the lowest binding energy were determined to be the most advantageous. To ascertain the optimal ligand binding poses and evaluate the orientations of the ligands within the binding site, a thorough analysis of the docking results was conducted using a Discovery Studio Visualizer 2021. This analysis yielded valuable information regarding the affinity of the ligands for the receptor and the interactions between the ligands and the receptor.

Molecular dynamic simulation

Molecular dynamics simulations were performed in Gromacs 2023 to investigate the interactions between ligands and the DPP4 macromolecule24,25,26. The simulations were conducted for 100 nanoseconds to ensure a thorough examination of the ligand-macromolecule interactions over time.[24]

For the preparation of protein, pdb2gmx was used. To ionize the solution, NaCl was used, and the pH was measured. Particle-Mesh Ewald (PME) and Fast Fourier Transform (FFT) were utilized in the development of a periodic Boundary Condition system. The force fields of Amber99sb were utilized for the preparation of the ligands, and they

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were applied to the protein22, alongside the TIP3P water model, incorporating hydrogen mass repartitioning23.
Throughout the 100-ns simulation, data acquisition occurred at consistent intervals to assess critical metrics such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), and energy components. Simulations elucidated the stability and kinetics of ligand binding to the DPP4 macromolecule, facilitating a comprehensive investigation of the interactions and potential conformational alterations in the protein-ligand complex.

Results and Discussion

Drug likeness and Lipinski evaluations
Ligand data for compounds from *Laminaria japonica* were obtained from the Knapsack database at http://knapsack3d.sakura.ne.jp/.[18] The ligands were assessed based on Lipinski's five rules to determine their druglikeness and potential bioavailability. Out of the 39 ligands, 20 were found to meet all of Lipinski's criteria, which include a molecular weight of less than 500 Da, an octanol-water partition coefficient (LogP) of less than 5, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors. These 20 ligands were considered suitable for further analysis due to their favorable characteristics, suggesting their potential as effective drug-like compounds. Adherence to Lipinski's rules indicated that these ligands were likely to possess good oral bioavailability, making them promising candidates for subsequent molecular docking studies (Table 1).
By adhering to these guidelines, which assess factors like molecular weight, hydrogen bonding potential, and lipophilicity, it was ensured that the selected compounds had drug-like properties. This step was crucial in confirming the suitability of the ligands for molecular docking studies, increasing the likelihood of identifying compounds with promising bioactivity and good oral bioavailability. Narrowing down the selection to those that passed these rigorous criteria laid a solid foundation for the next stages of research, focused on evaluating their binding interactions and therapeutic potential.

Molecular docking
The docking validation process for the interaction of *Laminaria japonica* ligands with DPP4 was performed using AutoDock 4.2. The validation involved redocking the native ligand (PF2) to the active site of the DPP4 structure (PDB ID: 3F8S). The precision of this approach was assessed using the root mean square deviation (RMSD) between the re-docked conformation and the reference crystallographic structure, which should ideally be under 2 Å. By systematically modifying the grid center, the RMSD was optimized to 1.236 Å, thereby confirming the integrity of the docking configuration (Figure 1).

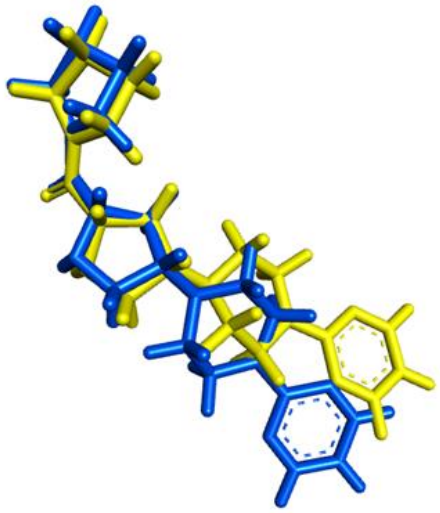

Figure 1: Visualization of re-docking for the re-docking of the native ligand (PF2) to the active site of the DPP4 structure (PDB ID: 3F8S). Overlaying of the native ligand X-ray crystallography (blue) and native ligand resulting from re-docking (yellow)

In the docking set-up, particular settings were utilized to enhance the grid box and search space. The dimensions of the grid box were established at 50 × 50 × 50 points, with a grid spacing of 0.375 Å. Grid center coordinates were accurately set at 11.161, 23.341, and 32.381, focusing on the active site DPP4. The genetic method utilized a population size of 100, achieving moderate precision to optimize both computational efficiency and accuracy. These parameters were chosen to guarantee that the docking simulations explored sufficiently the conformational space of the ligands.

Table 1: Drug likeness properties for ligands of *Laminaria japonica*

Ligand	Molecular Weight	Hydrogen Donor	Hydrogen Acceptor	Log P	Molar Refractivity	TMS
Hexanal*	100	0	1	1.765	30.205	0
L-Fucose*	164	4	5	-2.193	34.574	0
D-Mannitol	182	6	6	-3.585	38.198	1
Linoleic Acid	280	1	2	5.884	86.993	1
Myristic acid*	228	1	2	4.772	68.713	0
Oleic acid	282	1	2	6.108	87.087	1
Palmitic acid	256	1	2	5.552	77.947	1
Palmitoleic acid	254	1	2	5.328	77.853	1
2-Heptanone*	114	0	1	2.155	34.822	0
Cholesterol	386	1	1	7.388	119.052	1
Fucosterol	412	1	1	7.944	128.192	1
Benzeneacetaldehyde*	120	0	1	1.428	36.209	0
1,4-Cineole*	154	0	1	2.744	45.526	0
D-Galactose	180	5	6	-3.221	35.985	1

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(2*E*)-Octenal*	126	0	1	2.321	39.345	0
(2*E*)-2-Nonenal*	140	0	1	2.711	43.962	0
1-Octen-3-ol*	128	1	1	2.113	40.345	0
Beta-Ionone*	192	0	1	3.514	60.082	0
Benzaldehyde*	106	0	1	1.499	31.829	0
Heptanal*	114	0	1	2.155	34.822	0
2-Hexen-1-ol*	100	1	1	1.335	31.133	0
Laminine*	188	2	3	-1.059	49.732	0
Matsutake alcohol*	128	1	1	2.113	40.345	0
1-Octen-3-one*	126	0	1	2.321	39.345	0
2-Octen-1-ol*	128	1	1	2.115	40.367	0
2(4H)-Benzofuranone*	134	0	2	1.313	36.014	0
Isoquinoline*	129	0	1	2.234	41.742	0
Trans-2-undecen-1-ol*	170	1	1	3.285	54.218	0
Laminarin	504	11	16	-7.572	101.252	4
Fucoidan	158	5	7	-2.179	33.181	1
Fucoxanthin	658	2	6	8.692	194.104	2
Violaxanthin	600	2	4	8.969	183.213	2
Zeaxanthin	568	2	2	10.547	184.171	2
Astaxanthin	596	2	4	8.905	184.951	2
Tetradeconoic acid	270	0	2	5.639	82.305	1
Hexadeconoid acid	298	0	3	5.209	87.334	1
Guluronic acid	194	5	7	-3.129	36.535	1
Mannuronic acid	194	5	7	-3.129	36.535	1
β-Sitosterol	414	1	1	8.024	128.216	1

* Ligands that met all of Lipinski's criteria for drug-likeness

Upon validation, a minimum binding energy of -9.21 kcal/mol was attained, which signifies a robust interaction between the ligand and the active site of DPP4. The calculated inhibitory constant (K_i) was 176.32 nM, further indicating the potential of the ligand as a lead molecule for anti-type 2 diabetes. The low RMSD value of 1.236 Å validates the efficacy of the docking approach, indicating that the interactions between the ligand and DPP4 are highly reliable for subsequent *in silico* investigation.

The binding energy and inhibition constant (K_i) derived from molecular docking experiments provide essential insights into the capacity of several *Laminaria japonica* ligands to interact with DPP4 as leading molecules for antitype 2 diabetes (Table 2 and Fig. 2). Compared to the native ligand (PF2) of PDB ID 3F8S, which exhibited a binding energy of -9.21 kcal/mol and a Ki of 0.176 nM, most of the evaluated ligands demonstrated lower binding affinities and inhibition constants. PF2 serves as a reference, establishing a high standard for comparison, as its substantial binding energy signifies a consistent interaction with DPP4, and its exceptionally low Ki value indicates powerful inhibition. Among the highest-performing ligands, fucoidan, beta-ionone, and (2*E*)-octenal are notable, however, none exceed PF2 in binding affinity. Fucoidan demonstrated a binding energy of -5.79 kcal/mol and a Ki of 56.78 nM, indicating a moderately robust interaction with DPP4; yet, it remains inferior to PF2 in terms of binding strength and inhibitory efficacy. Beta-ionone, with a binding energy of -5.64 kcal/mol and a Ki of 73.19 nM, and (2*E*)-octenal, with a binding energy of -5.60 kcal/mol and a Ki of 82.98 nM, indicate substantial inhibition, although less powerful than the native ligand.

On the contrary, additional ligands including 1-octen-3-one and 2(4H)-benzofuranone exhibited favorable binding energies of -5.18 kcal/mol and -5.17 kcal/mol, respectively, along with K_i values of 160.66 nM and 162.44 nM. These ligands, although they show marginally reduced binding affinity compared to the leading candidates, nevertheless possess adequate inhibition constants that suggest promise as mild inhibitors of DPP4. Their interactions with the enzyme indicate that they may be promising secondary candidates for additional examination. On comparison of these results with PF2, it is evident that the native ligand is the most effective for binding energy and Ki, while several *Laminaria japonica* compounds exhibit potential as competing inhibitors. Fucoidan, beta-ionone, and (2*E*)-octenal warrant further refinement or optimization; nevertheless, their binding energies and inhibition constants indicate that further alterations may be required to achieve the efficacy of PF2.

The molecular docking analysis of *Laminaria japonica* ligands with DPP4 provides essential information on their binding affinities and hydrogen-bond interactions with significant active site residues, notably Ser630 and Glu205.[25] PF2, the endogenous ligand of DPP4, demonstrated the highest binding energy of -9.21 kcal/mol, establishing six hydrogen bonds, including interactions with Ser630, Glu205, and Ser209. These interactions are essential as Ser630 and Glu205 are recognized for their significant role in the catalytic activity of DPP4. The many hydrogen bonds of PF2 enhance its strong binding, as evidenced by its exceptional inhibitory constant (K_i) of 0.176 nM. PF2 functions as a powerful inhibitor, establishing the reference standard for the assessment of other ligands.

Multiple ligands exhibited favorable binding affinities; however, none exceeded PF2. Fucoidan, exhibiting a binding energy of -5.79 kcal/mol and forming seven hydrogen bonds, emerged as a prominent candidate. Although it did not interact directly with Ser630 or Glu205, it established hydrogen bonds with residues such as Phe578, Gln606, and

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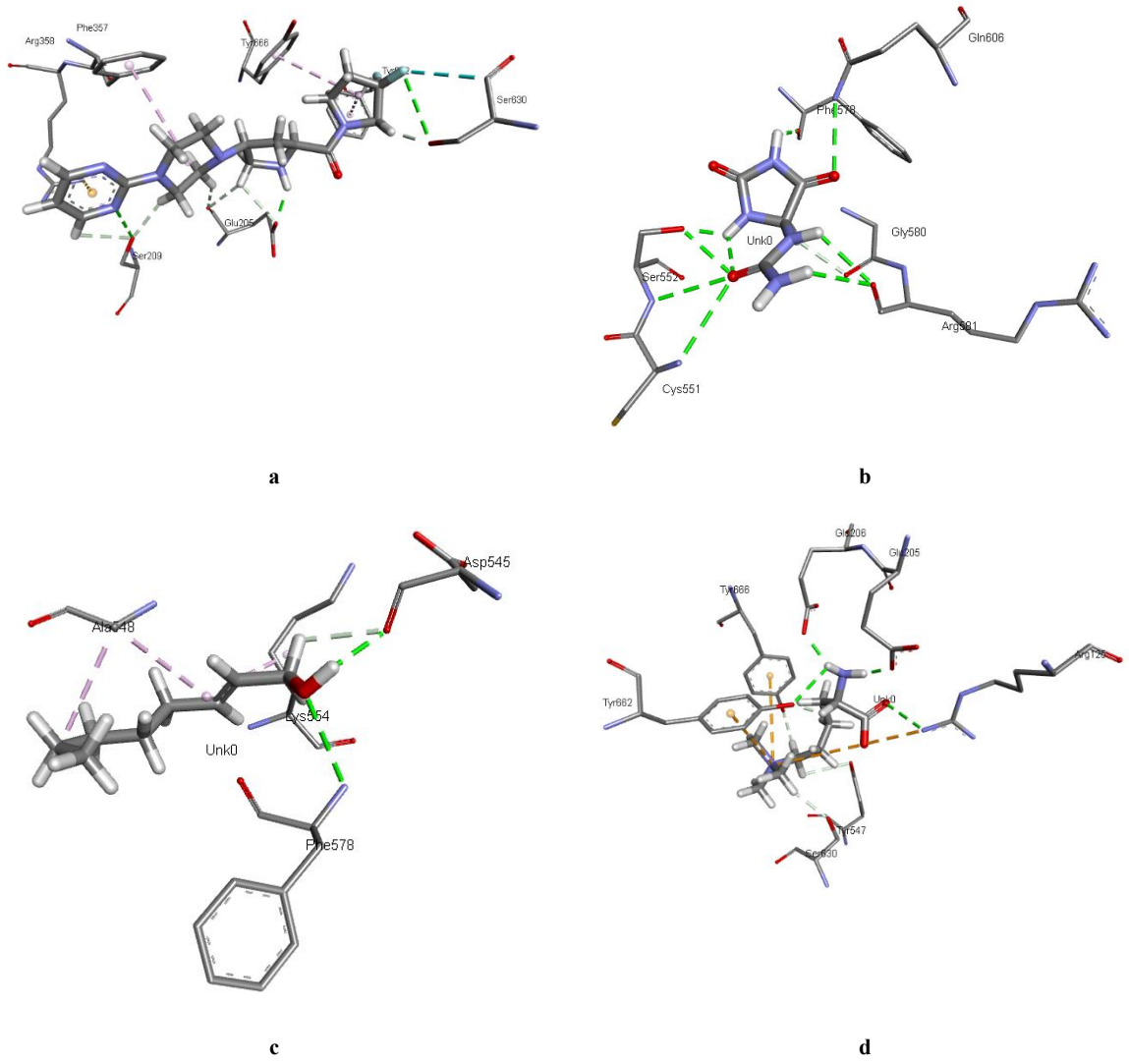

Figure 2: 3D interaction for best poses docked of three best ligands. The native ligand (a), fucoidan (b), beta-ionone (c), and (2E)-octenal (d).

Ser552, which are crucial for maintaining the ligand within the active site. The hydrogen bonding network and the binding energy indicate that fucoidan may serve as an effective inhibitor, but less potent than PF2, due to its absence of direct contact with the critical residues Ser630 and Glu205 essential for catalytic inhibition. Beta-ionone, which exhibits a binding energy of -5.64 kcal/mol, demonstrated an effective equilibrium between the binding affinity and hydrogen bonding interactions. Establish two hydrogen bonds with His740 and Arg125, none of which is integral to the essential residues Ser630 and Glu205. However, its binding energy and K_i of 73.19 nM indicate that it remains a viable moderate inhibitor of DPP4. Laminine, which exhibits a binding energy of -5.6 kcal/mol, established four hydrogen bonds, notably one with Glu205, suggesting its capacity to engage with the enzyme's active site. This contact amplifies its inhibitory capacity since Glu205 is directly implicated in the catalytic mechanism of DPP4, making laminine a compelling ligand for further investigation.

Various ligands, including 1-octen-3-one and (2*E*)-octenal, displayed modest binding affinities (-5.18 kcal/mol and -5.6 kcal/mol, respectively) but showed restricted hydrogen bonding interactions. For example, 1-octen-3-one did not establish any hydrogen bonds, which may explain its decreased inhibitory potential. (2*E*)-Octenal established a solitary hydrogen bond with Phe578, which is distant from the residues of the critical active site, indicating that it may be less effective in inhibiting DPP4 compared to leading agents such as fucoidan and beta-ionone.

In summary, although PF2 is the most effective ligand for binding energy and interactions with essential residues Ser630 and Glu205, many *Laminaria japonica* ligands, including fucoidan, beta-ionone, and laminine, exhibit considerable potential as DPP4 inhibitors. The direct interaction between laminine and Glu205 is particularly significant, as it underscores the need to focus on crucial residues at the active site to improve the binding affinity and inhibitory potential. Subsequent research may concentrate on the refinement of these ligands to improve their interaction with Ser630 and Glu205, thus potentially increasing their inhibitory efficacy against DPP4.

Molecular dynamics

RMSD

The root mean square deviation (RMSD) study (Fig. 3) of insights into the flexibility of individual amino acid residues of the macromolecule for understanding the stability and conformational changes caused by ligand interaction, which will aid in the design and optimization of prospective medicinal drugs[26]. The root mean square deviation (RMSD) study of the macromolecule (PDB ID 3F8S) and its native ligand (PF2) during 100 ns of molecular dynamics simulation offers significant insights into the structural stability and flexibility of the system (Fig. 3a). The RMSD values for the macromolecule varied from 0.19 nm at

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the beginning of the simulation to 0.63 nm at 100 ns, indicating substantial flexibility during the simulation. Initially, there was a significant increase in RMSD in the first 10 ns, reaching 0.64 nm, indicating an initial structural modification. After 20 ns, the macromolecule's RMSD stabilized between 0.36 and 0.73 nm, indicating structural stability post-equilibration.

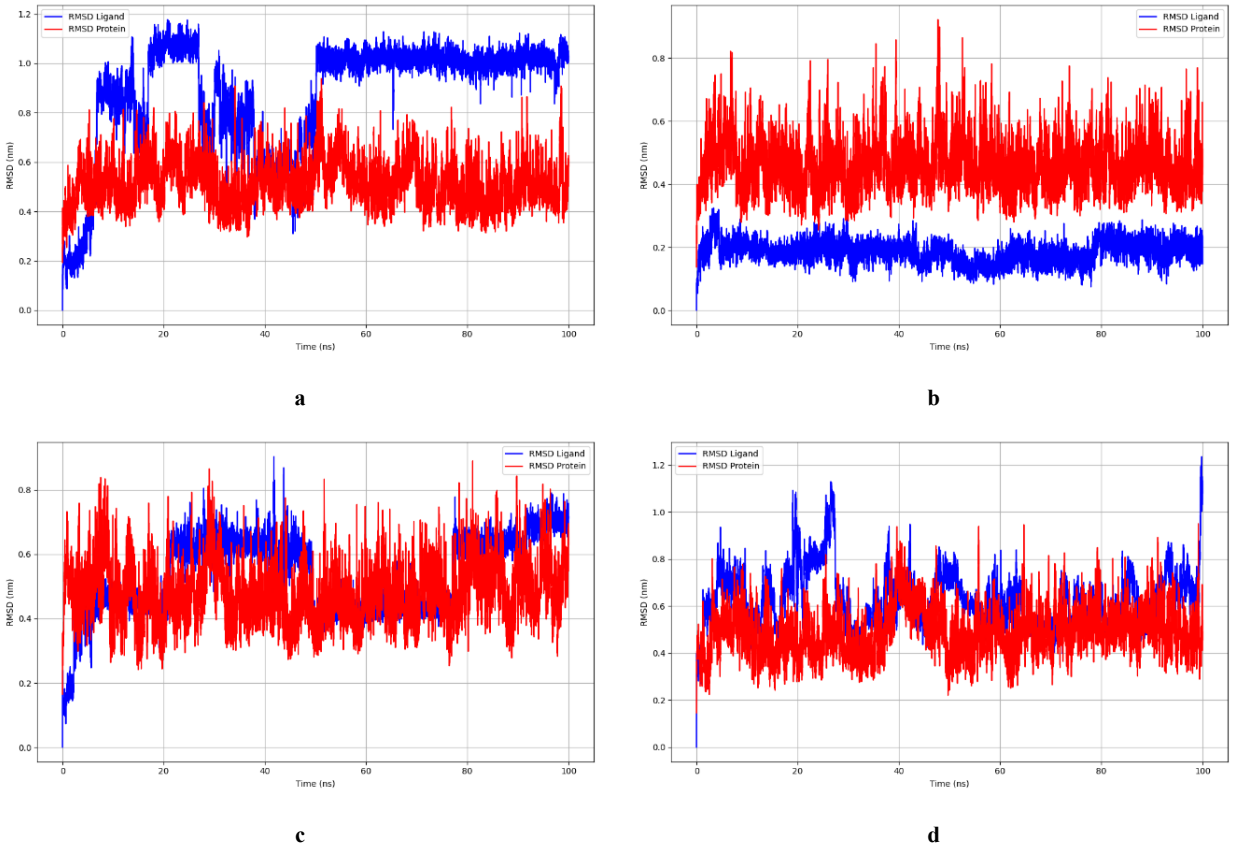

Figure 3: RMSD for best poses docked of three best ligands. The native ligand (a), fucoidan (b), beta-ionone (c), and (2E)-octenal (d). Protein and ligand were colored in blue and red, respectively.

On the contrary, the native ligand (PF2) exhibited a pronounced fluctuation in RMSD during the simulation, beginning at 0.0005 nm at 0 ns and rapidly increasing to 1.07 nm by 25 ns. This increased fluctuation signifies enhanced flexibility and mobility of the ligand inside the binding pocket, potentially suggesting modifications in its binding orientation to facilitate interactions with adjacent residues. Despite this variability, PF2 exhibited a consistent pattern of RMSD increases and decreases, indicating possible structural alterations or binding reconfigurations during its interaction with DPP4. This behavior is anticipated, as ligands typically demonstrate greater flexibility than macromolecules in simulations.

Upon comparison of the RMSD profiles of the macromolecule and PF2, the macromolecule demonstrated relative stability beyond the initial adjustment period, while PF2 persisted in showing greater variations. At 100 ns, PF2 had an RMSD of 1.03 nm, which indicates continuous motion, while the macromolecule maintained a constant value of 0.63 nm. This indicates that the macromolecule attained a stable configuration early in the simulation, while PF2 underwent more significant structural rearrangements within the active region. This ligand flexibility may be related to the pursuit of the best binding configuration or adaptation to alterations in the protein structure.

The RMSD findings demonstrate that the macromolecule attained stability, whereas the native ligand (PF2) exhibited flexibility and dynamism within the active site. The preliminary structural modifications noted for both the macromolecule and the ligand indicate that the system reached equilibrium between approximately 20 and 30 nanoseconds.

The RMSD study of the macromolecule (PDB ID 3F8S) and fucoidan throughout a 100 ns molecular dynamics simulation provides insights into their structural stability and interaction dynamics[22,27,28] (Fig. 3b). The macromolecule DPP4 began with a RMSD of 0.14 nm at 0 ns and displayed oscillations over time, stabilizing between 0.33 nm and 0.62 nm throughout the simulation. The most significant increases transpired at around 15 ns and 35 ns, indicating instances of structural reorganization. However, these mutations persisted within an acceptable range, signifying that the protein predominantly preserved its structural integrity. At 100 ns, the RMSD value of 0.43 nm indicates that the macromolecule maintained general stability during the simulation, although there were modest conformational alterations.

Fucoidan, the ligand being studied, demonstrated a distinct RMSD pattern. Commencing with an almost negligible RMSD of 0.0005 nm, fucoidan exhibited a progressive enhancement in flexibility, attaining its initial apex of 0.23 nm at 5 ns. In particular, fucoidan exhibited a more consistent RMSD profile between 10 ns and 55 ns, ranging from 0.14 to 0.23 nm, suggesting that the ligand underwent fewer structural alterations than the macromolecule. This indicates that fucoidan quickly established a favorable binding orientation within the active site of DPP4 and maintained this configuration with few modifications. Toward the conclusion of the simulation, fucoidan's RMSD exhibited a modest rise, attaining 0.25 nm at 95 ns and stabilizing at approximately 0.23 nm by 100 ns, indicating a degree of mobility while maintaining overall stability within the binding pocket.

Analysis of the RMSD profiles for the macromolecule and fucoidan reveals that DPP4 demonstrated superior flexibility throughout the simulation compared to the ligand. The macromolecule had substantial structural changes, particularly at 15 ns and 35 ns, probably attributable to movements of the protein side chains or alterations in the backbone near the binding site.

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Table 2: Binding energy and binding mode properties for ligands of *Laminaria japonica*

Ligand	Binding energy (kcal/mol)	Inhibition constant, Ki (μM)	Number of hydrogen bonds (HB)
PF2	-9.21	0.176	6 HB with A:Ser209**, A:Glu205, A:Ser630**
Hexanal	-4.63	406.34	2 HB with A:Tyr547, A:Ala548
L-Fucose	-4.09	998.10	7 HB with A:Tyr547, A:Tyr631, A:Tyr666, A:Tyr662, **A:Ser630**
Myristic acid	-4.5	501.29	2 HB with A:Trp629, A:Lys554
2-Heptanone	-5.02	209.73	2 HB with A:Gln606, A:Gly580
Benzeneacetaldehyde	-5.03	204.87	1 HB with A:Tyr547
1,4-cineole	-5.47	97.41	0 HB
D-galactose	-4.39	601.35	6 HB with A:Tyr70, A:Lys71, A:Ser59
(2*E*)-Octenal	-5.60	82.98	1 HB with A:Phe578
(2*E*)-2-nonenal	-5.32	126.14	1 HB with A:Phe578
1-Octen-3-ol	-3.97	1,230	1 HB with A:Tyr547
Beta-lonone	-5.64	73.19	2 HB with A:His740, A:Arg125
Benzaldehyde	-4.59	428.69	1 HB with A:Ala548
Heptanal	-4.75	331.92	1 HB with A:Glu91
2-Hexen-1-ol	-4.78	315.08	1 HB with A:Phe578
Laminine	-5.60	79.11	4 HB with A:Glu206, **A:Glu205**, A:Arg125, A:Tyr662
Matsutake alcohol	-5.03	206.91	2 HB with A:Asp579, A:Ser552
1-Octen-3-one	-5.18	160.66	0 HB
2-Octen-1-ol	-5.67	70.20	2 HB with A:Asp579, A:Phe578
2 (4H) -benzofuranone	-5.17	162.44	1 HB with A:Lys71
Isoquinoline	-4.96	232.91	0 HB
Trans-2-undecen-1-ol	-4.08	1,030	1 HB with A:Leu57
Fucoidan	-5.79	56.78	7 HB with A:Cys551, A:Phe578, A:Gln606 A:Arg581, A:Ser552

On the contrary, Fucoidan had a comparatively stable conformation, indicating a robust interaction with DPP4. This stability indicates that fucoidan was effectively integrated within the active site and did not require significant structural modifications to preserve its binding. The tiny variations in fucoidan's RMSD towards the conclusion of the simulation are anticipated, as ligands frequently undergo slight conformational alterations while investigating various binding modalities.

RMSD statistics indicate that both the macromolecule and fucoidan preserved structural integrity during the 100-ns simulation. DPP4 exhibited slight flexibility, presumably due to inherent protein dynamics, while fucoidan had a stable binding profile with minimal movement after the establishment of its first binding orientation. The steady interaction profile of fucoidan indicates that it is a suitable option for further investigation, as its consistency within the active site implies a significant binding affinity and potential effectiveness as a lead chemical for the inhibition of DPP4 in antitype 2 diabetes therapy.

The RMSD study of the macromolecule (PDB ID 3F8S) and beta-ionone during a 100 ns molecular dynamics simulation elucidates the structural stability and behavior of both the protein and the ligand (Fig. 3c). The macromolecule DPP4 had an RMSD of around 0.16 nm at 0 ns, demonstrating variations during the simulation. Initially, the RMSD exhibited a consistent rise, reaching a maximum of around 0.64 nm at 30 ns, subsequently fluctuating between 0.30 nm and 0.74 nm for the duration of the experiment. The oscillations indicate that the macromolecule experienced substantial structural changes, perhaps due to interactions with beta-ionone or the inherent flexibility of the protein. Despite these alterations, RMSD remained within an acceptable range, indicating that DPP4 preserved its overall structural integrity.

Beta-ionone, the ligand being studied, began with an almost negligible RMSD of 0.0005 nm, indicating its first docking conformation within the active site DPP4. The RMSD of the ligand escalated abruptly to approximately 0.44 nm within the initial 5 ns and subsequently maintained relative stability with minor changes until 25 ns. From 25 ns onward, beta-ionone exhibited a significant increase in flexibility, with its RMSD reaching 0.70 nm at 25 ns and then oscillating between 0.40 nm and 0.72 nm for the duration of the experiment. This pattern indicates that beta-ionone examined many conformations inside the binding site, presumably modifying its location in reaction to interactions with critical residues of DPP4. The elevated RMSD values near the end of the simulation suggest that beta-ionone failed to sustain a fully stable binding conformation and experienced some dynamic fluctuations within the active site.

On a comparison of the RMSD profiles of the macromolecule and beta-ionone, it is evident that both the protein and the ligand had variations during the simulation; however, the macromolecule demonstrated superior overall stability. The significant increase in beta-ionone RMSD after 25 ns may indicate a more flexible binding mode, in which the ligand explored many conformations within the active site. This flexibility may suggest weaker connections or the capacity of beta-ionone to adjust to the protein's changing environment. In contrast, DPP4 exhibited a more stable structural profile, with intermittent

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conformational modifications likely reflecting alterations in binding interactions with beta-ionone.

RMSD findings reveal that DPP4 preserved its structural integrity during the simulation, but beta-ionone showed greater variability in its binding conformation. This indicates that the beta-ionone interaction with DPP4 may be less rigid than that of certain other ligands, potentially affecting its binding affinity and overall stability as a candidate for DPP4 inhibition. However, the relatively steady RMSD values for both the macromolecule and beta-ionone in the latter phase of the simulation indicate that the ligand successfully sustained a stable association with DPP4, although some conformational modifications were required to achieve this stability.

The RMSD study of the macromolecule (PDB ID 3F8S) and laminine over a 100 ns molecular dynamics simulation elucidates the structural stability and interaction dynamics between the protein and the ligand (Fig. 3d). The macromolecule DPP4 displayed a RMSD beginning at 0.15 nm, with significant oscillations during the simulation, reaching a maximum of roughly 0.73 nm at around 40 ns. The variations suggest that the protein had multiple structural changes during the simulation, perhaps due to its dynamic characteristics or modifications in response to interactions with laminine. After the simulation at 100 ns, the RMSD for DPP4 stabilized around 0.54 nm, indicating that although the protein underwent some structural rearrangements, it predominantly preserved its structural integrity.

Laminine, the ligand of interest, began with an RMSD of 0.0004 nm, indicating its first docking conformation. However, the ligand exhibited a more fluctuating RMSD profile in contrast to that of the macromolecule. Laminine's RMSD exhibited a marked increase to 0.71 nm at 5 ns, followed by substantial fluctuations during the simulation, culminating in a maximum of 0.81 nm at 25 ns. This diversity indicates that laminine underwent significant structural alterations and modifications within the DPP4 binding region. The ligand's RMSD decreased to 0.53 nm at 60 ns, thereafter rising to 1.08 nm at 100 ns. This trend suggests that laminine persistently investigated various conformations within the binding pocket over the simulated time.

A comparison of RMSD profiles between DPP4 and laminine indicates that the macromolecule had overall structural stability with periodic oscillations, while laminine showed increased variability in its binding conformation. The significant variations in laminine RMSD indicate that the ligand can often alter its binding mode to enhance interactions with the protein. This flexibility may signify either a less stable binding relationship or the ligand's capacity to adjust to various conformational states within the binding pocket. On the contrary, the stable RMSD of the macromolecule indicates that DPP4 maintained a consistent overall structure despite slight conformational variations.

The RMSD findings indicate that laminine interacts dynamically with DPP4, exhibiting notable changes in its binding pose, and suggesting a flexible relationship. The macromolecule exhibited stability with intermittent structural modifications, whereas laminine's fluctuating RMSD indicates that it is experiencing conformational alterations to accommodate the binding environment. This versatility may suggest a robust yet adaptive interaction, potentially positioning laminine as a suitable option for further exploration as a lead chemical for DPP4 inhibition. Laminine's ability to modify its conformation within the binding pocket may enhance its effectiveness as a treatment agent for type 2 diabetes.

The RMSD analysis of PF2, fucoidan, beta-ionone, and laminine offers significant information on the stability and dynamics of their interactions with DPP4. PF2, the endogenous ligand, demonstrates the most stable association with DPP4, preserving a somewhat uniform binding conformation throughout the simulation. The RMSD values for both the ligand and macromolecule exhibit relatively minor variations, signifying a resilient and stable binding mode. Stability indicates that PF2 is adept at sustaining its interaction with DPP4, presumably due to its optimized binding within the active site.

On the contrary, fucoidan, beta-ionone, and laminine exhibit considerable heterogeneity in their interactions with DPP4. Fucoidan exhibits significant variations in its binding conformation, potentially indicating a less stable relationship relative to PF2. Beta-ionone demonstrates significant fluctuations in its RMSD as well as in that of the macromolecule, signifying a dynamic and adaptable binding method. Laminine exhibits the greatest variability, characterized by frequent and substantial fluctuations in RMSD, indicating a highly flexible but potentially less stable relationship. These distinctions underscore the diverse interaction dynamics of these ligands, offering insight into their potential as therapeutic agents targeting DPP4 for the treatment of type 2 diabetes.

RMSF

The root mean square fluctuation (RMSF) study elucidated the flexibility and dynamic behavior of the macromolecule in complex with several ligands,[29] such as PF2, fucoidan, beta-ionone, and laminine (Fig. 4). In PF2, the RMSF values for different residues demonstrated a comparatively steady interaction with the DPP4 macromolecule. Residues 39, 97, 98, and 99 exhibited considerable variability, indicating that PF2 maintained a stable binding conformation while allowing flexibility in certain areas of the protein. The equilibrium between stiffness and flexibility was essential to maintain a stable yet adjustable interaction with the target protein.

Fucoidan demonstrated elevated RMSF values in multiple residues, including 84, 85, 242, 243, and 244. Increased flexibility indicated that fucoidan caused greater conformational alterations in the DPP4 protein during the simulation. Residues 242 to 244 exhibited the most pronounced variations, suggesting that fucoidan binding may have substantially impacted these areas, potentially modifying the protein's structure and function. The elevated RMSF values at these locations indicated that fucoidan's interaction with DPP4 was more dynamic, perhaps influencing the stability of its binding.

Beta-ionone exhibited significant fluctuations in RMSF, especially in residues 84, 97, 242, 243, and 244, similar to fucoidan. The increased flexibility observed in these residues indicated that beta-ionone may have caused significant structural alterations in the DPP4 protein. This dynamic behavior suggested a less permanent binding relationship to PF2, as the ligand may have induced considerable alterations in the protein structure during the simulation. Nevertheless, the overall RMSF values for beta-ionone were somewhat worse than those for fucoidan, indicating a relatively more stable, yet still flexible interaction.

Laminine demonstrated RMSF values comparable to those of beta-ionone, with notable changes detected in residues 84, 97, 242, 243, and 244. Elevated RMSF values in these areas indicated that laminine, similar to beta-ionone, imparted significant flexibility to the structure of the DPP4 protein. The increased flexibility in crucial residues such as 242 and 243 may have influenced protein activity and stability, potentially affecting the efficacy of laminine as a primary chemical. Upon comparison, PF2 demonstrated the most stable interaction with DPP4, while fucoidan, beta-ionone, and laminine exhibited variable levels of flexibility that may have impacted their therapeutic potential.

RMSF analysis indicated that fucoidan, beta-ionone, and laminine significantly enhanced the flexibility of the DPP4 protein, with considerable variations observed in critical residues including 84, 97, 242, 243, and 244. Fucoidan demonstrated elevated RMSF values, signifying significant conformational alterations and possible instability in the protein structure. Beta-ionone induced notable dynamic behavior, albeit with marginally reduced RMSF values, indicating a relatively more stable interaction. Laminine exhibited the highest RMSF values on several residues, signifying more pronounced structural disturbances. The investigation indicated that all three ligands influenced the flexibility of the protein, with laminine and fucoidan eliciting a more pronounced dynamic behavior than beta-ionone, potentially affecting their efficacy as therapeutic agents for type 2 diabetes.

Energy component

The analysis of the energy components of the ligands PF2, fucoidan, beta-ionone, and laminine, derived from molecular dynamics simulations using Gromacs 2023, yielded significant insights into their interactions with DPP4 (Fig. 5). PF2, the endogenous ligand, demonstrated the most advantageous energy profile with a total energy of -25.59 kcal/mol. This was mainly attributable to its robust van der Waals interactions (ΔVDWAALS = -33.52 kcal/mol) and a well-

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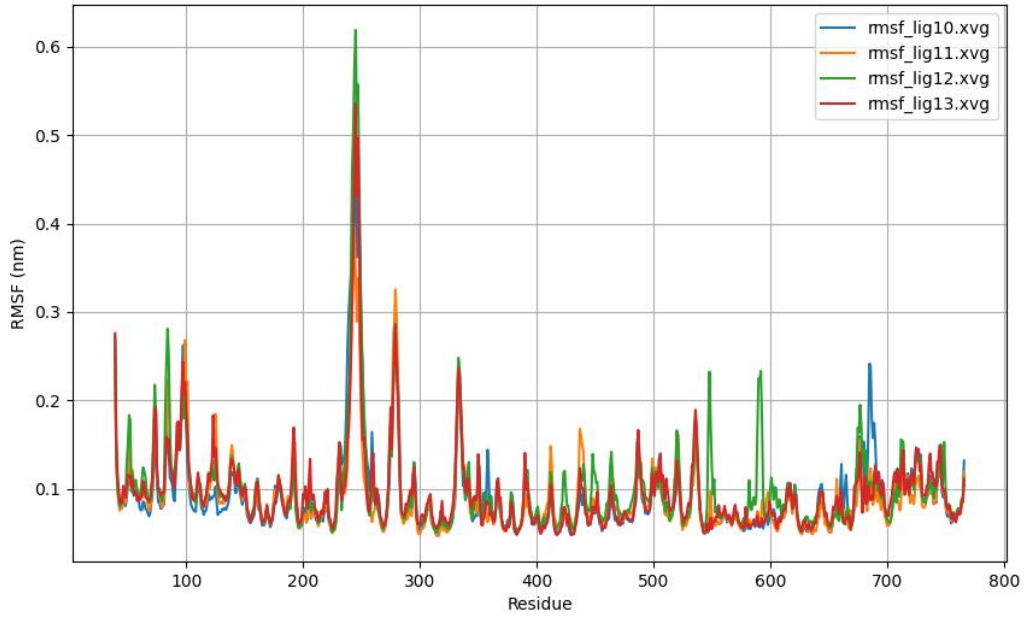

Figure 4: RMSF for best poses docked of three best ligands. The native ligand, fucoidan, beta-ionone, and (2E)-octenal were colored in blue, orange, green, and red, respectively.

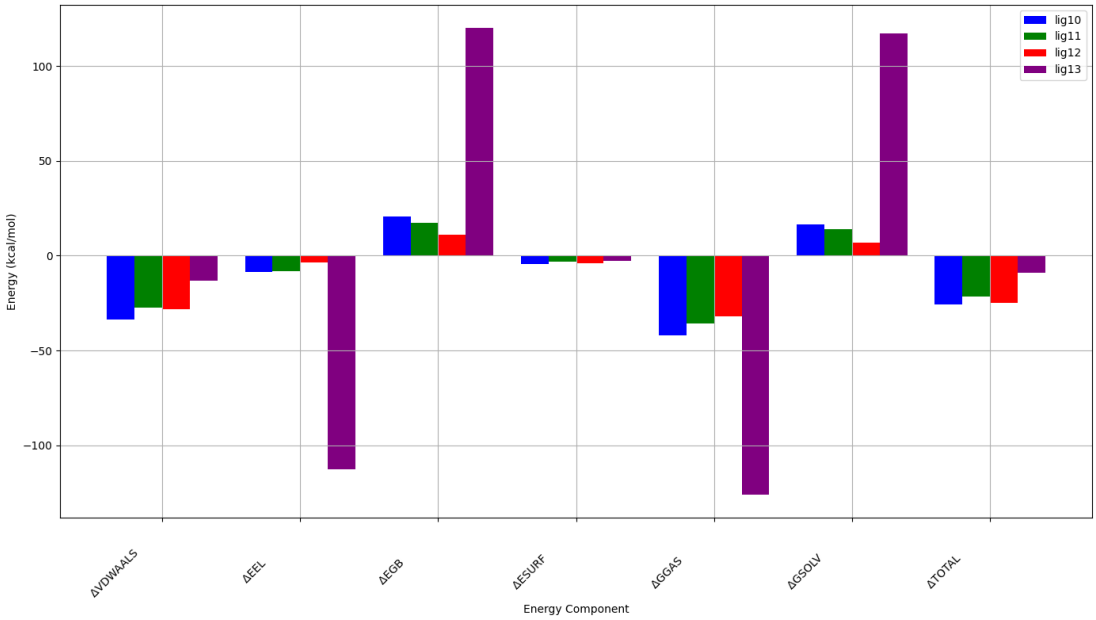

Figure 5: Energy components for best poses docked of three best ligands. The native ligand, fucoidan, beta-ionone, and (2E)-octenal were colored in blue, green, red, and purple, respectively.

balanced electrostatic energy (ΔEEL = -8.46 kcal/mol). However, PF2 had a relatively elevated free energy (ΔGSOLV = 16.39 kcal/mol), suggesting that although it established strong bonds with the protein, it faced significant solvation penalties.

Fucoidan had a suboptimal overall energy profile with a total energy of -21.49 kcal/mol. The van der Waals interactions were lower than those of PF2 (ΔVDWAALS = -27.56 kcal/mol), and the electrostatic energy was somewhat less negative (ΔEEL = -8.1 kcal/mol). Fucoidan had a higher solvation free energy (ΔGSOLV = 14.16 kcal/mol) although it remained more favorable in this aspect compared to beta-ionone. The ΔEGB contribution of EGB (17.24 kcal/mol) demonstrated a moderate stabilizing impact resulting from electrostatic interactions with the solvent.

Beta-ionone had a total energy of -24.73 kcal/mol, marginally surpassing fucoidan but being less advantageous than PF2. The van der Waals interactions were mild (ΔVDWAALS = -28.11 kcal/mol),

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whereas the electrostatic energy was considerably less favorable (ΔEEL = -3.66 kcal/mol) in comparison to PF2. The free energy of solvation was lower (ΔGSOLV = 7.04 kcal/mol), indicating that although beta-ionone exhibited satisfactory binding interactions, it underwent comparatively less stabilizing effects of solvation relative to the other ligands. Laminine demonstrated the least advantageous total energy of -8.82 kcal/mol, characterized by a significantly suboptimal van der Waals interaction score (ΔVDWAALS = -13.37 kcal/mol) and markedly detrimental electrostatic interactions (ΔEEL = -112.55 kcal/mol). The elevated free solvation energy of Laminine (ΔGSOLV = 117.1 kcal/mol) substantially impacted its overall unfavorable energy profile. Although laminine exhibits robust electrostatic stabilization in the solvent, its interaction with DPP4 is less energetically favorable than that of PF2, fucoidan, and beta-ionone. This research highlighted the disparities in binding efficiencies and stabilities between ligands, which may affect their viability as lead molecules in the development of antitype 2 diabetes therapeutics.

Molecular dynamics simulations and molecular docking investigations for PF2, fucoidan, beta-ionone, and laminine yielded a thorough understanding of their energetic profiles. PF2 exhibited the most advantageous binding energy from the docking at -9.21 kcal/mol and a cumulative energy of -25.59 kcal/mol of the energy components of the molecular dynamics. This demonstrated that PF2 not only exhibited a great affinity for DPP4 but also maintained a steady connection during the simulation. Fucoidan exhibited a significant binding energy of -5.79 kcal / mol by docking, although it demonstrated a less advantageous total energy of -21.49 kcal/mol in molecular dynamics simulations. This indicated that although fucoidan exhibited a considerable affinity for DPP4, its overall stability and interaction energy were inferior to those of PF2. Beta-ionone exhibited a binding energy of -5.64 kcal/mol from docking and an overall energy of -24.73 kcal/mol from molecular dynamics analysis. The data indicated that beta-ionone had a marginally lower binding affinity than fucoidan, although it maintained relatively stable contact with DPP4 during the simulation. Laminine had the most unfavorable binding energy of -5.60 kcal/mol and an even less advantageous total energy of -8.82 kcal/mol. This suggested that laminine had a reduced binding affinity for DPP4 and showed much weaker stability in its association compared to the other ligands.[8] The research indicated that PF2 exhibited excellent binding and stability, whereas fucoidan and beta-ionone displayed moderate effectiveness and laminine revealed the least favorable energetic profile.

Conclusion

The *in silico* screening of *Laminaria japonica* ligands as potential inhibitors of DPP4 for type 2 diabetes treatment provided valuable insights. Among the ligands analyzed fucoidan, beta-ionone, and laminin showed the most promising binding affinities for DPP4, positioning them as potential inhibitors for type 2 diabetes treatment. Although these ligands exhibited lower binding energies than the native ligand PF2, beta-ionone stood out due to its superior ΔTOTAL value in molecular dynamics simulations, suggesting it may be the most effective candidate for further investigation. This study highlights the potential of natural compounds as therapeutic agents for diabetes management. In a future perspective, experimental validation of these ligands, particularly beta-ionone, *in vitro* and *in vivo* will be crucial.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Candler T, Mahmoud O, Lynn R, Majbar A, Barrett T, Shield J. Continuing rise of type 2 diabetes incidence in children and young people in the UK. Diabet Med 2018; 35: 737–744.
2. Balaji R, Duraisamy R, Kumar M. Complications of diabetes mellitus: A review. Drug Invent Today 2019; 12: 98.
3. Andary R, Fan W, Wong ND. Control of cardiovascular risk factors among US adults with type 2 diabetes with and without cardiovascular disease. Am J Cardiol 2019; 124: 522–527.
4. Kumar MS, Sharma SA. Toxicological effects of marine seaweeds: a cautious insight for human consumption. Crit Rev Food Sci Nutr 2021; 61: 500–521.
5. Lee I-S, Ko S-J, Lee YN, Lee G, Rahman MH, Kim B. The effect of *Laminaria japonica* on metabolic syndrome: A systematic review of its efficacy and mechanism of action. Nutrients 2022; 14: 3046-3066.
6. Celcia Gnana Rathinam W, Bragadeeswaran S, Kumaresan S, Gunamathy K, Visnu B, Mohamed Asarudeen J, Sasidharan T, Srikavibharathi S. Marine Resources: A Sustainable and Promising Source for Cosmetic Industries. In: Multidisciplinary Applications of Marine Resources: A Step towards Green and Sustainable Future. Springer, 2024; 103–140.
7. Zhang C, Jia J, Zhang P, Zheng W, Guo X, Ai C, Song S. Fucoidan from *Laminaria japonica* ameliorates type 2 diabetes mellitus in association with modulation of gut microbiota and metabolites in streptozocin-treated mice. Foods 2022; 12: 33-51.
8. Deacon CF. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. Front Endocrinol 2019; 10: 80-94.
9. Saini K, Sharma S, Khan Y. DPP-4 inhibitors for treating T2DM-hype or hope? an analysis based on the current literature. Front Mol Biosci 2023; 10: 1130625-1130644.
10. Yang N, He L-Y, Liu P, Li Z-Y, Yang Y-C, Ping F, Xu L-L, Li W, Zhang H-B, Li Y-X. Dipeptidyl peptidase-4 inhibitors and the risk of infection: A systematic review and meta-analysis of cardiovascular outcome trials. World J Diabetes 2024; 15: 1011-1020.
11. Dowarah J, Singh VP. Anti-diabetic drugs recent approaches and advancements. Bioorg Med Chem 2020; 28: 115263-115278.
12. Singh S, Baker QB, Singh DB. Molecular docking and molecular dynamics simulation. In: Bioinformatics. Elsevier, 2022; 291–304.
13. Huey R, Morris GM, Forli S. Using AutoDock 4 and AutoDock vina with AutoDockTools: a tutorial. Scripps Res Inst Mol Graph Lab 2012; 10550: 1000-1032.
14. Kutzner C, Kniep C, Cherian A, Nordstrom L, Grubmüller H, de Groot BL, Gapsys V. GROMACS in the cloud: A global supercomputer to speed up alchemical drug design. J Chem Inf Model 2022; 62: 1691–1711.
15. Snyder HD, Kucukkal TG. Computational chemistry activities with Avogadro and ORCA. J Chem Educ 2021; 98: 1335–1341.
16. Jejurikar BL, Rohane SH. Drug designing in discovery studio. 2021
17. Ammirati MJ, Andrews KM, Boyer DD, Brodeur AM, Danley DE, Doran SD, Hulin B, Liu S, McPherson RK, Orena SJ. (3, 3-Difluoro-pyrrolidin-1-yl)-[(2S, 4S)-(4-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrrolidin-2-yl]-methanone: A potent, selective, orally active dipeptidyl peptidase IV inhibitor. Bioorg Med Chem Lett 2009; 19: 1991–1995.
18. Nakamura K, Shimura N, Otabe Y, Hirai-Morita A, Nakamura Y, Ono N, Ul-Amin MA, Kanaya S. KNApSAcK-3D: a three-

ISSN 2616-0684 (Print)
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dimensional structure database of plant metabolites. Plant Cell Physiol 2013; 54: e4-e12.
19. Karami TK, Hailu S, Feng S, Graham R, Gukasyan HJ. Eyes on Lipinski's rule of five: A New "rule of thumb" for physicochemical design space of ophthalmic drugs. J Ocul Pharmacol Ther 2022; 38: 43–55.
20. Lim VT, Hahn DF, Tresadern G, Bayly CI, Mobley DL. Benchmark assessment of molecular geometries and energies from small molecule force fields. F1000Research 2020; 9: 1390-1412.
21. Asnawi A, Nedja M, Febrina E, Purwaniati P. Prediction of a Stable Complex of Compounds in the Ethanol Extract of Celery Leaves (*Apium graveolens* L.) Function as a VKORC1 Antagonist: http://.www.doi.org/10.26538/tjnpr/v7i2.10. Trop J Nat Prod Res TJNPR 2023; 7: 2362–2370.
22. Yuliantini A, Ocktavyanie S, Febrina E, Asnawi A. Virtual Screening Using a Ligand-based Pharmacophore Model from Ashitaba (*Angelica keiskei* K.) Isolates and Molecular Docking to Obtained New Candidates as α-Glucosidase Inhibitors: http://www.doi.org/10.26538/tjnpr/v8i1.15. Trop J Nat Prod Res TJNPR 2024; 8: 5811–5819.
23. A. Asnawi, Aman LO, Nursamsiar, A. Yuliantini, E. Febrina. Molecular Docking and Molecular Dynamic Studies: Screening Phytochemicals of *Acalypha Indica* Against Braf Kinase Receptors For Potential Use In Melanocytic Tumours. Rasayan J Chem 2022; 15: 1352–1361.
24. Febrina E, Asnawi A. Lead compound discovery using pharmacophore-based models of small-molecule metabolites from human blood as inhibitor cellular entry of SARS-CoV-2. J Pharm Pharmacogn Res 2023; 11: 810–822.
25. Mathur A, Feng S, Hayward JA, Ngo C, Fox D, Atmosukarto II, Price JD, Schauer K, Märtlbauer E, Robertson AAB, Burgio G, Fox EM, Leppla SH, Kaakoush NO, Man SM. A multi-component toxin from Bacillus cereus incites inflammation and shapes host outcome via the NLRP3 inflammasome. Nat Microbiol 2019; 4: 362–374.
26. Hussain S, Iqbal A, Hamid S, Putra PP, Ashraf M. Identifying alkaline phosphatase inhibitory potential of cyclooxygenase-2 inhibitors: Insights from molecular docking, MD simulations, molecular expression analysis in MCF-7 breast cancer cell line and *in vitro* investigations. Int J Biol Macromol 2024; 6: 132721-132736.
27. Ijoma I, Okafor C, Ajiwe V. Computational Studies of 5-methoxypsolaren as Potential Deoxyhemoglobin S Polymerization Inhibitor. Trop J Nat Prod Res TJNPR 2024; 8: 8835–8841.
28. Rahman H, Bintang MI, Asnawi A, Febrina E. Exploring the Molecular Interactions between Volatile Compounds in Coconut Shell Liquid Smoke and Human Bitter Taste TAS2R46 Based on the Molecular Docking and Molecular Dynamics: http://www.doi.org/10.26538/tjnpr/v7i12.31. Trop J Nat Prod Res TJNPR 2023; 7: 5587–5594.
29. Alaofi AL. Exploring structural dynamics of the MERS-CoV receptor DPP4 and mutant DPP4 receptors. J Biomol Struct Dyn 2022; 40: 752–763.