J Herbmed Pharmacol. 2025; 14(4): 514-523.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2025.52928

Journal of Herbmed Pharmacology

Exploring marine natural compounds: *In silico* investigation of *Halimeda tuna* natural compounds for PPAR-γ targeting in type 2 diabetes

Aiyi Asnawi[1*], Ellin Febrina[2], Widhya Aligita[3], Nina Nurafiani Putri[1], Anne Yuliantini[1], Deden Indra Dinata[1], Hamidah Rahman[4], Purnawan Pontana Putra[5]

[1]Department of Pharmacochemistry, Faculty of Pharmacy, Universitas Bhakti Kencana, Jl. Soekarno-Hatta No. 754, Bandung 40617, Indonesia
[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km. 21, Jatinangor 45363, Indonesia
[3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Bhakti Kencana, Jl. Soekarno-Hatta No. 754, Bandung 40617, Indonesia
[4]Department of Public Health, Faculty of Health Sciences, Universitas Muhammadiyah Maluku Utara, Jl. KH. Ahmad Dahlan No. 100 Ternate 97719, North Maluku, Indonesia
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Andalas, Padang, 25163, Indonesia

ARTICLE INFO

Article Type:
Short Communication

Article History:
Received: 27 Jan. 2025
Revised: 26 Jun. 2025
Accepted: 16 Jul. 2025
epublished: 1 Oct. 2025

Keywords:
Halimeda tuna
In silico
PPAR-γ inhibition
Type 2 diabetes

ABSTRACT

Introduction: The peroxisome proliferator-activated receptor gamma (PPAR-γ) is a crucial regulator of glucose metabolism and insulin sensitivity, making it a primary target for diabetes therapy. *Halimeda tuna*, a marine alga, has shown potential as a source of bioactive chemicals with possible anti-diabetic effects. However, there is little information on the particular interactions of *H. tuna*-derived natural compounds with PPAR-γ. This research aimed to find prospective lead compounds by molecular docking and to evaluate their stability and interaction dynamics with the PPAR-γ using molecular dynamics simulations.

Methods: The natural compounds were downloaded from http://knapsack3d.sakura.ne.jp/, then refined based on Lipinski's criteria, and optimized for molecular docking. The PPAR-γ macromolecule (PDB ID 1I7I) was also refined to retain only the receptor and its native natural compound (AZ2). Molecular docking was carried out using AutoDock 4.2 to analyze the binding energy and binding mode. To evaluate the stability of the natural compound-receptor complexes, molecular dynamics simulations were conducted for 100 ns using Gromacs 2023.

Results: The molecular docking studies showed that the binding energies for M12 and M7 were -10.77 kcal/mol and -9.91 kcal/mol, respectively. However, when molecular dynamics (MD) simulations were conducted, the total energy values became more negative, with M12 showing -48.25 kcal/mol and M7 -40.13 kcal/mol.

Conclusion: M12 (2-desoxypleniradin-L-|A-arabinopyranoside, 2-acetate) appears to be a promising candidate as a potential PPAR-γ inhibitor for treating type 2 diabetes.

Implication for health policy/practice/research/medical education:
This study demonstrated the potential of discovering anti-diabetic lead compounds from *Halimeda tuna*, including M12, using molecular docking and their stability and interaction dynamics through molecular dynamics (MD) simulations. The results suggested that these techniques might be useful in identifying promising natural compounds for PPAR-γ, which could serve as lead compounds for T2DM treatment. This highlighted the importance of incorporating computational tools into research practices to accelerate drug discovery, reduce costs, and improve the precision of identifying bioactive molecules. Furthermore, the findings encouraged further exploration of marine bioactive compounds, potentially expanding the scope of natural products in the search for novel therapeutic agents.

Please cite this paper as: Asnawi A, Febrina E, Aligita W, Putri NN, Yuliantini A, Dinata DI, et al. Exploring marine natural compounds: *In silico* investigation of *Halimeda tuna* natural compounds for PPAR-γ targeting in type 2 diabetes. J Herbmed Pharmacol. 2025;14(4):514-523. doi: 10.34172/jhp.2025.52928.

***Corresponding author**: Aiyi Asnawi,
Email: aiyi.asnawi@bku.ac.id

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has been steadily increasing worldwide, with the International Diabetes Federation (IDF) reporting that over 537 million adults were living with the condition in 2021, a figure that is expected to rise to 784 million by 2045 (1). This surge is largely driven by factors such as aging populations, sedentary lifestyles, and rising obesity rates, which are closely linked to the development of T2DM. The growing burden of T2DM underscores the urgent need for novel therapeutic strategies, especially those that target the underlying mechanisms of insulin resistance and glucose metabolism. One potential intervention involves targeting the peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor crucial for regulating glucose metabolism, insulin sensitivity, and adipogenesis. PPAR-γ is a promising target for T2DM therapy due to its essential role in enhancing insulin sensitivity and regulating adipose tissue function, both of which are often compromised in T2DM (2).

Unlike other diabetes targets, such as glucagon-like peptide-1 (GLP-1) receptors or sodium-glucose cotransporter-2 (SGLT-2) inhibitors, PPAR-γ activation addresses multiple underlying factors of T2DM, including insulin resistance and dysregulated fat metabolism. Additionally, PPAR-γ agonists like thiazolidinediones have proven effective in improving insulin sensitivity. However, these therapies can be associated with side effects, including weight gain and fluid retention (2).

Halimeda tuna, a marine green algae, has gained significant interest for its potential medicinal benefits (3). This macroalgae is known for its diverse bioactive components, which have been studied for various pharmacological activities, including anti-inflammatory, antioxidant, and anti-diabetic properties (4). Investigating marine compounds, such as those found in *H. tuna*, as therapeutic agents offers a promising alternative to traditional terrestrial sources. The ocean is a vast, largely untapped resource of bioactive molecules, and marine compounds often exhibit unique characteristics compared to their terrestrial counterparts, including distinct chemical structures and mechanisms of action (5).

Marine bioactive compounds, especially those from *H. tuna*, are frequently adapted to the extreme and varied conditions of the ocean, allowing them to possess novel bioactivities not typically found in land-based organisms. These compounds tend to have complex structures that enable more selective and potent interactions with biological targets (6). In the context of T2DM, marine-derived compounds, such as those from *H. tuna*, offer potential advantages over terrestrial sources, including greater specificity in targeting key receptors like PPAR-γ and fewer side effects (7). However, despite the promising potential of *H. tuna*-derived chemicals, their specific interactions with crucial therapeutic targets like PPAR-γ (8) have not been thoroughly examined. This gap in molecular understanding limits the ability to design and enhance these molecules for more effective therapeutic applications. In drug screening, molecular docking and molecular dynamics (MD) serve as complementary tools that offer detailed insights into the stability of drug-target interactions (9-11). Molecular docking is used to predict the binding of small molecules to a target protein by evaluating various binding conformations and scoring their affinity (12,13). MD simulations provide a dynamic perspective by modelling the time-dependent behaviour of the protein-natural compound complex, allowing researchers to evaluate the stability of the binding, conformational changes, and the overall flexibility of the complex (14,15). The main purpose of this study was to perform an *in-silico* analysis of *H. tuna* natural compounds to forecast their interactions with PPAR-γ as a candidate inhibitor for T2DM. This was achieved by the utilization of molecular docking and MD simulations to assess the binding affinity, stability, and interaction dynamics of various natural compounds with the receptor.

Materials and Methods

Hardware

The computational work for this study was conducted using a high-performance computer system running on Linux Ubuntu 22.04 LTS. The system was equipped with an Intel Xeon Quad Core E5 2690 processor running at 2.13 GHz, a GeForce RTX 3060 graphics card with 8 GB of memory, a 1 TB storage disk, and 32 GB of RAM. To perform the simulations and analyses, AutoDock 4.2 was employed for molecular docking and GROMACS for MD simulations (16), and Avogadro for geometry optimization. The docking results were visualized using Discovery Studio Visualizer (DSV).

Preparation of the target protein

In this investigation, the macromolecule was synthesized using PDB ID 1I7I, essential for PPAR-γ, retrieved from the Protein Data Bank at https://www.rcsb.org/. The downloaded file originally comprised many components, including water molecules, heteroatoms, and supplementary natural compounds. The file was rigorously refined to concentrate exclusively on the macromolecule and its native ligand, AZ2. This technique entailed eliminating all non-essential components while preserving only the crucial macromolecular structure and AZ2.

Preparation of test natural compounds

In this work, the natural compounds were prepared by initially downloading the natural compound data in *.mol format from *H. tuna* from the website (https://pubchem.ncbi.nlm.nih.gov/taxonomy/170433#section=Organism-Disease-Co-Occurrences-in-Literature) as well as from

several previously published studies (17,18). Each natural compound was filtered according to Lipinski's guidelines (19) to ensure its relevance and eligibility for further investigation. Only natural compounds that adhered to Lipinski's criteria, encompassing molecular weight, lipophilicity, and hydrogen bond interactions, were chosen for the investigation. The chosen natural compounds were subjected to geometry optimization to enhance their molecular structures. The optimization was carried out using the MMFF94 force field in Avogadro.

Molecular docking simulation

The docking validation approach to evaluate the interaction of *H. tuna* natural compounds with PPAR-γ was performed using AutoDock 4.2 (20,21). The native ligand AZ2 was first re-docked at the active site of the PPAR-γ structure with PDB ID 1I7I. The precision of the docking validation was assessed by computing the root mean square deviation (RMSD) between the redocked conformation and the reference crystallographic structure. The objective of the approach was to achieve an RMSD of less than 2 Å to ensure the dependability of the docking protocol. Subsequent to validation, the molecular docking simulation of the test natural compounds was executed utilizing the confirmed docking protocol. The natural compounds were docked into the macromolecular structure of PPAR-γ, identified by PDB ID 1I7I, utilizing gridbox parameters derived during the validation step. The docking parameters comprised a grid spacing of 0.375 Å, a population size of 100, and a moderate mutation rate of 2. This configuration facilitated an extensive examination of the natural compound binding sites and their interactions with the macromolecule, yielding comprehensive insights into their possible binding affinities and processes.

Molecular dynamics simulations

MD simulations were performed using GROMACS 2023 for 100 nanoseconds (ns) to explore the interactions between *H. tuna* natural compounds and the PPAR-γ receptor. The process began with system preparation, which involved solvation and ionization to create an appropriate environment for the natural compound-receptor complexes. A cubic box was used to surround the system with water molecules (TIP3P model), and sodium and chloride ions were added to neutralize the system, simulating physiological conditions. After setting up the system, energy minimization was carried out using the steepest descent method to remove any steric clashes and ensure system stability. The simulations were then run for 100 ns at a constant temperature of 310 K and pressure of 1 bar. To improve simulation efficiency, a time step of 2 fs was used, and the SHAKE algorithm constrained bond lengths involving hydrogen atoms. Long-range electrostatic interactions were calculated with the Particle Mesh Ewald (PME) method for accurate modelling. After completing the simulations, data analysis was conducted to assess the stability of the natural compound-receptor interactions using parameters like root mean square deviation (RMSD), root mean square fluctuation (RMSF), and energy components. These analyses helped evaluate the binding stability and conformational changes of the natural compounds.

Results

Molecular docking

To ensure the drug-likeness of the 20 natural compounds from *H. tuna*, the compounds were filtered using Lipinski's Rule of Five. After applying natural compound filtration criteria based on Lipinski's rule of five, which assesses factors like molecular weight, lipophilicity, and hydrogen bond donors/acceptors, 18 natural compounds were found to meet the necessary criteria. These 18 natural compounds were then selected for molecular docking simulations to assess their potential interactions with PPAR-γ, a key target for T2DM management. The binding energy and inhibition constant of the *H. tuna* natural compounds were evaluated by molecular docking with AutoDock to examine their interaction with PPAR-γ (Table 1).

Validation for the docking procedure via redocking of AZ2 was performed using grid points at X: 56, Y: 50, Z: 56, with an off-site spacing of 0.375 and grid center coordinates at X: 21.677, Y: 23.38, Z: 13.884. The RMSD of the redocked AZ2 was found to be 1.341 Å, indicating a reliable alignment and confirming the accuracy of the docking procedure. In the binding mode, analysis provided valuable insights into binding energy, hydrogen bonding (HB), and the interaction with key amino acid residues (Table 1 and Figure 1). The docking results showed varying levels of affinity for PPAR-γ, as indicated by the binding energy (ΔG) values, which ranged from -9.39 kcal/mol to -10.77 kcal/mol, and the inhibition constants (Ki), which reflected the binding strength in micromolar concentrations.

Root mean square deviation

The MD simulation of PDB ID 1I7I and its native ligand (AZ2), performed with GROMACS 2023 for 100 ns, involved an analysis of the RMSD (22) to evaluate the stability of the macromolecule and natural compound over time (Figure 2).

Root mean square fluctuation

The RMSF for the two natural compounds, AZ2, M12, and M7, exhibited different levels of flexibility in MD simulations (Figure 3).

Molecular mechanics generalized Born surface area

Molecular mechanics generalized Born surface area

Table 1. Binding energy and binding mode properties of *Halimeda tuna* natural compounds based on molecular docking analysis, with the rank-1 cluster representing the lowest energy conformation

Natural compound	Code	Binding energy, ΔG (kcal/mol)	Inhibition constant, Ki (μM)	Hydrogen bond interactions
AZ2*	NL	-9.39	0.13	Ser83, Tyr267, His243, Gly78, Ile75
Metformin**	RD	-4.28	730.18	Phe76, His117, Ser83, Tyr267
Dodecanoic acid	M1	-5.22	150.40	Tyr267, His117, Ser83
Amaralin	M2	-8.12	1.11	Cys79
Heleniamarin	M3	-7.99	1.38	Tyr121, Ser83, Tyr267
(1S,5R)-5-[(1R,2S)-2-[(1E)-2,6-dimethylhepta-1,5-dienyl]-1-formylcyclopropyl]cyclopent-2-ene-1,2-dicarbaldehyde	M4	-8.94	0.28	Arg74, Gly59, Lys59
(1R,2R,6R,7R,9S,12R,13S,15R)-13-hydroxy-2,7,12,13-tetramethyl-10,14-dioxatetracyclo [7.5.1.02,6.012,15] pentadec-4-ene-3,11-dione	M5	-7.75	2.80	Ser136
(3aR,5R,5aR,8aR,9R,9aS)-9-hydroxy-5,8a-dimethyl-1-methylidene-3a,4,5,5a,9,9a-hexahydroazuleno[6,7-b]furan-2,8-dione	M6	-8.66	0.45	Lys59
(2'S,4R,4aR,7aR)-2'-[(1E)-2,6-dimethylhepta-1,5-dienyl]-1-oxospiro [3,4a,5,7atetrahydrocyclopenta [c]pyran-4,1'-cyclopropane]-7-carbaldehyde	M7	-9.91	0.05	Tyr267
(3aS,5aS,8R,8aR,9aR)-5,8-dimethyl-1-methylidene-8-[(2S,3R,4S,5S)-3,4,5-trihydroxyoxan-2-yl]oxy-5a,6,7,8a,9,9a-hexahydro-3aH-azuleno[6,5-b]furan-2-one	M8	-7.94	0.28	Lys59, Ile75, Cys79
[(1R,3aR,5R,8aR,9S,9aR)-1,5,8a-trimethyl-2,8-dioxo-3a,4,5,7,9,9a-hexahydro-1H-azuleno[6,5-b]furan-9-yl] acetate	M9	-8.88	0.31	Arg74, Lys59, Gly78
(1R,2S,3S,7S,10S,11R,13S,14R)-2,14-dihydroxy-1-methyl-4-methylidene-6,12dioxatetracyclo [8.4.0.03,7.011,13]tetradecan-5-one	M10	-6.95	8.90	Lys59, Gly78
4-[(1R,2S)-3,3-dimethyl-2-(3-oxobutyl)cyclobutyl]pent-4-enal	M11	-7.70	2.28	Thr62, His60
2-Desoxypleniradin-L-|A-arabinopyranoside, 2-acetate	M12	-10.77	0.01	Lys59, Ile78, Cys79, Ser136
[(1S,2R,3R,7S,9R,10S,11R,13S,14R)-14-hydroxy-1,9-dimethyl-4-methylidene-5-oxo-6,12-dioxatetracyclo [8.4.0.03,7.011,13]tetradecan-2-yl] acetate	M13	-7.62	2.60	Arg82, Ser136
(1R,2R,6S,7S,9R,12R,13R,15R)-13-hydroxy-2,7,12,13-tetramethyl-10,14-dioxatetracyclo[7.5.1.02,6.012,15] pentadec-4-ene-3,11-dione	M14	-7.99	1.38	Ile75, Lys59
4-[(1S,4R)-4-(5-hydroxypent-1-en-2-yl)-2,2-dimethylcyclobutyl]butan-2-one	M15	-7.45	3.46	Lys57, Ile56, His60, Glu53, Lys59
Dibutyl phthalate	M16	-7.12	6.04	-
Thiophanate-methyl	M17	-6.92	8.45	Leu134, Ser136, Phe58, Ile75, Arg74, Cys79
Carbofuran	M18	-6.78	10.67	Gly78, Lys59

*AZ2: (2*S*)-2-ethoxy-3-[4-(2-{4-[(methylsulfonyl)oxy]phenyl}ethoxy)phenyl]propanoic acid is the native ligand of PDB ID 1I7I.
**: RD is the reference drug.
NL: Native ligand.

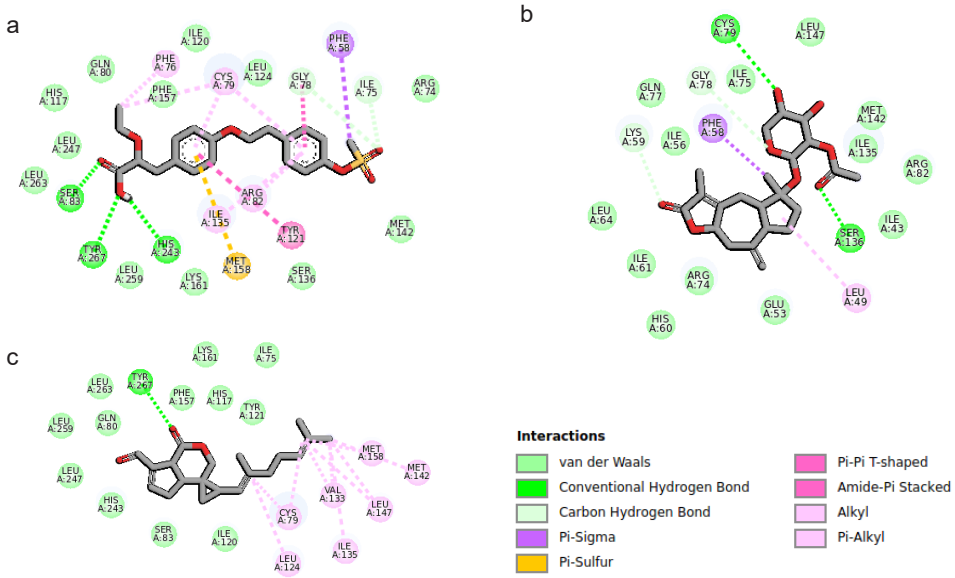

Figure 1. 2D visualization of the best docked poses for the top two natural compounds. (a) Native ligand, (b) M12, and (c) M7. The best docked poses were visualized using Discovery Studio Visualizer, with the rank-1 cluster representing the conformation with the lowest energy.

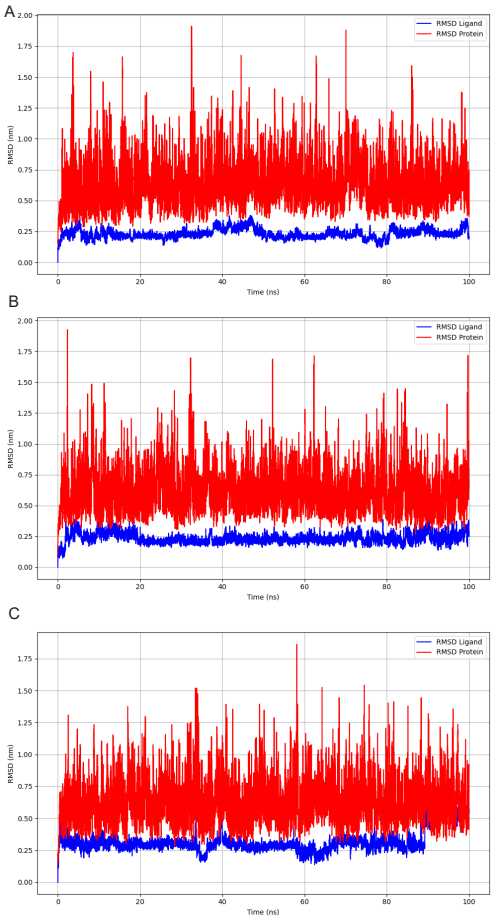

Figure 2. Root mean square deviation (RMSD) for the best docked poses of the top two natural compounds. (a) Native ligand, (b) M12, and (c) M7. The RMSD values were calculated to assess the stability and consistency of the natural compound-receptor interactions over 100 ns. The natural compounds and PPAR-γ were colored in blue and red, respectively.

(MMGBSA) was a method used to estimate the binding free energy of a natural compound-receptor complex by combining molecular mechanics (MM) energy with solvation effects using the generalized Born (GB) model and surface area (SA) terms. These components were summed to calculate the total binding energy, providing an estimate of how strongly a natural compound bound to its receptor, factoring in both enthalpic and non-polar solvation contributions. This method was widely used due to its computational efficiency and ability to estimate binding affinities without needing explicit solvent molecules.

In the MD simulation of natural compounds interacting with PPAR-γ, the energy components were assessed for the NL, M12, and M7 (Figure 4).

Discussion

PPAR-γ was selected for this study due to its critical role in regulating glucose and lipid metabolism, making it a key target for the treatment of T2DM (23). PPAR-γ activation improves insulin sensitivity, reduces inflammation, and enhances adipogenesis, all of which are essential in managing T2DM (24). Additionally, marine algae, specifically *H. tuna*, have demonstrated promising properties in previous studies, such as antioxidant, anti-inflammatory, and anti-diabetic effects (25). The combination of PPAR-γ's biological significance and *H. tuna*'s therapeutic potential made them a compelling subject for investigation in this context.

The native ligand (AZ2) of PDB ID 1I7I was used as a benchmark for comparison (23). AZ2 demonstrated a binding energy of -9.39 kcal/mol and an inhibition constant (Ki) of 0.13 μM, signifying a robust interaction

with the receptor. This established a standard for evaluating the effectiveness of *H. tuna* natural compounds in targeting PPAR-γ as prospective agents for anti-T2DM pharmaceuticals.

The second-most effective natural compound, M12, had the highest binding affinity among *H. tuna* natural compounds, with a binding energy of -10.77 kcal/mol and an exceptionally low inhibition constant of 0.01 μM. This natural compound outperformed AZ2 in both metrics, suggesting a potentially improved ability to interact with the PPAR-γ receptor. In contrast, the third most effective natural compound, M7, had a higher binding affinity than AZ2, with a binding energy of -9.91 kcal/mol and an inhibition constant of 0.05 μM. The results indicated that these natural compounds might surpass AZ2 in inhibiting PPAR-γ, rendering them intriguing lead compounds.

AZ2, with a binding energy of -9.39 kcal/mol and a dissociation constant (Ki) of 0.13 μM, formed five hydrogen bonds with crucial residues of PPAR-γ, including Ser83, Tyr267, His243, Gly78, and Ile75. These interactions indicated strong binding, particularly with Ser83 and Tyr267, which were a part of the receptor's natural compound binding pocket. The binding mode of the compound suggested a favourable alignment within the active site, enhancing its potential to modulate PPAR-γ function. The second compound, M12, exhibited a slightly stronger binding affinity with a ΔG of -9.91 kcal/mol and a Ki of 0.05 μM. It formed a single hydrogen bond with Tyr267, a key residue involved in receptor activation. This suggested that, despite the minimal hydrogen-bond interaction, the compound likely achieves effective binding through other molecular interactions within

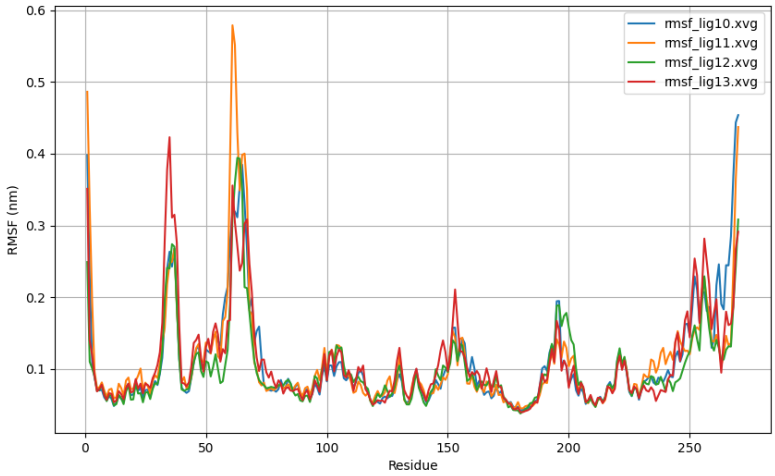

Figure 3. The root mean square fluctuation (RMSF) for the best poses docked of the two best natural compounds and the native ligand (NL). The NL, M12, and M7 were colored in blue, orange, and green, respectively. The RMSF values were calculated to assess the stability and consistency of the natural compound-receptor interactions over 100 ns.

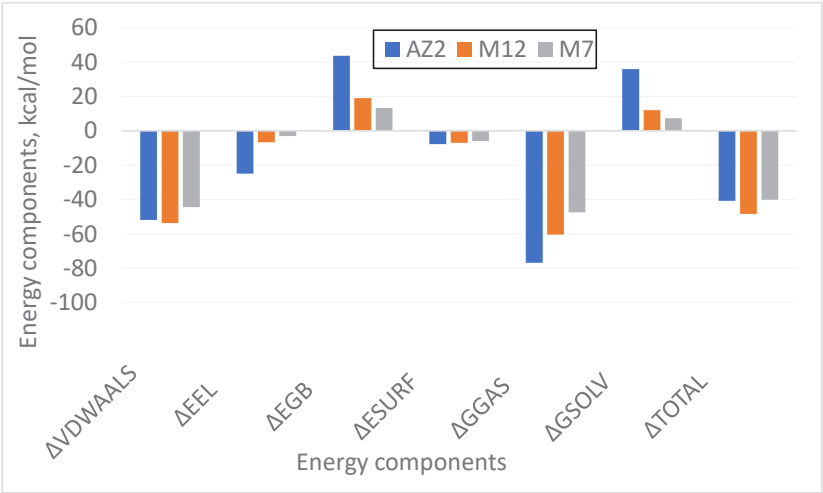

Figure 4. Energy components for the best docked poses of the top two natural compounds. Native ligand, M12, and M7 are colored in blue, orange, and grey, respectively. The energy components were calculated using GMX_MMPBSA to evaluate the binding interactions and stability of the natural compound-receptor complexes.

the binding pocket. The third compound, M7, showed a binding energy of -10.77 kcal/mol, the strongest among the compounds tested, with a very low Ki value of 0.01 μM. This compound formed four hydrogen bonds with Lys59, Gly78, Cys79, and Ser136, highlighting interactions with some of PPAR-γ's critical catalytic residues, such as Gly78 and Cys79. These interactions were important as they suggested that this compound might stabilize the receptor-natural compound complex and potentially influence the receptor's catalytic activity, making it a promising candidate for further *in vivo* investigation. In comparison, the reference drug, metformin, showed weaker binding affinities with PPAR-γ. Metformin had a ΔG of -4.28 kcal/mol and formed four hydrogen bonds with residues such as Phe76, His117, Ser83, and Tyr267. However, despite these interactions, the binding affinities of the reference drugs were significantly lower than the compounds from *H. tuna*, suggesting that these marine-derived natural compounds might have a stronger potential for PPAR-γ targeting.

Crucial catalytic residues of PPAR-γ, including Gly78, Ser83, Tyr267, and His243, played an important role in the interaction with marine compounds and reference drugs (24). In particular, Gly78 and Ser83 were involved in the interactions of both main compounds, indicating their importance in stabilizing the natural compound-receptor complex. These findings emphasized the potential of *H. tuna*-derived natural compounds as more potent and specific modulators of PPAR-γ compared to traditional drugs such as metformin and acarbose.

Metformin was included in the docking process as the reference compound due to its established role in T2DM management (26). Although this drug did not directly target PPAR-γ, it was commonly used in the clinical treatment of T2DM, providing a comparative baseline to evaluate the efficacy of *H. tuna*-derived natural compounds. Metformin works primarily by reducing hepatic glucose production and improving insulin sensitivity, although its interaction with PPAR-γ remains indirect (26).

MD simulations were not conducted for metformin because its mechanisms of action and stable pharmacokinetics were well-established, so the focus was placed on novel marine compounds, where MD simulations could offer more insight into their binding stability and interactions with PPAR-γ.

This approach aimed to uncover new therapeutic possibilities by exploring the behaviour of these marine-derived compounds in greater detail. In particular, M12 formed four hydrogen bonds with key residues such as Lys59, Gly78, Cys79, and Ser136, indicating its potential to bind to the receptor and influence its catalytic activity. Despite differences in the binding interactions between M12 and AZ2, the former's strong binding affinity (ΔG = -10.77 kcal/mol, K_i = 0.01 μM) and interactions with crucial residues like Gly78 and Cys79 suggested that it might effectively modulate PPAR-γ activity, supporting its potential as a PPAR-γ inhibitor.

The AZ2 exhibited considerable early fluctuations, with RMSD values reaching a maximum of 0.296 Å at around 45 ns before stabilizing towards the conclusion of the experiment. The steady RMSD values, especially after 60 ns, signified a very persistent interaction with the macromolecule during the early adaption phase. Although there was some preliminary mobility, AZ2 demonstrated a consistently secure and reliable binding relationship.

M12 exhibited considerable RMSD oscillations initially during the simulation; however, these fluctuations began to settle around 40 ns, with RMSD values reaching a maximum of 0.354 Å by 90 ns. The natural compound demonstrated fluctuation, but a stable structure was achieved at the end of the simulation. The behaviour of RMSD indicated that this natural compound underwent significant conformational changes before achieving stability, suggesting a marginally weaker initial binding relative to AZ2.

M7 exhibited the most pronounced fluctuations in RMSD among the natural compounds, particularly after 40 ns, reaching a high RMSD value of 0.564 at 95 ns. The persistent fluctuation in RMSD values during simulation indicated a decreased stability of the interaction with the macromolecule. The elevated RMSD values of this natural compound indicated a potentially weaker and less consistent binding than those of the other natural compounds, suggesting a diminished overall stability of the interaction.

RMSF values indicated the variability of particular residues and natural compounds, providing insight into the flexibility of binding interactions (27). Compared to the amino acid residues Ser63 and Glu205, which were crucial for PPAR-γ interactions, the natural compounds exhibited minor variations in these areas, particularly near residue 61 for M12. This suggested that the flexibility of the natural compounds in these regions might influence their ability to bind stably to the target protein. The interaction of the natural compound with Ser63, along with other key residues such as Glu205, played an important role in determining the general binding affinity and stability of the natural compound-receptor complex.

In the analysis of RMSF relative to the RMSD of M12 and M7, M12 demonstrated elevated RMSF values, peaking at 0.5793 Å, signifying enhanced flexibility and possibly dynamic behavior in its interaction with the target protein. This variation might affect the stability of its binding affinity. The M7 exhibited reduced RMSF values, reaching a maximum of 0.3944 Å. This indicated a more stable connection with restricted flexibility, suggesting that the natural compound retained a fixed position within the binding site. This relative stability might lead to more reliable binding interactions, rendering it a promising

option for further exploration.

ΔTOTAL indicated that M12 had the highest negative total energy components of -48.25 kcal/mol, signifying its strong overall binding affinity. M7 exhibited a notable binding affinity with ΔTOTAL values of -40.13 kcal/mol. The findings highlighted M12 as the most promising option among the evaluated natural compounds for targeting PPAR-γ.

M12 was considered the most promising compound not only due to its favourable binding energy of -10.77 kcal/mol in the docking simulation but also due to its outstanding stability during MD simulations. The compound exhibited a strong binding affinity with a ΔG of -48.25 kcal/mol in the MD simulation, the most negative among all tested natural compounds. This substantial energy change indicated that M12 undergoes minimal structural fluctuations, reflecting its ability to form stable interactions with PPAR-γ over the course of the 100 ns simulation. Furthermore, the RMSD values of this compound remained relatively low throughout the simulation, suggesting that it maintained consistent and stable interactions within the receptor binding pocket.

The stability of M12 was further supported by its interactions with key residues in PPAR-γ, including Gly78, Cys79, and Ser136. These residues were critical for the function of the receptor, and their interactions with the natural compound suggested that M12 could effectively modulate PPAR-γ activity. The ability of the compound to form multiple stable interactions with these important residues, combined with its minimal structural deviation during MD simulation, positioned it as a highly promising candidate for further exploration in the treatment of T2DM. On the contrary, other natural compounds exhibited slightly less favourable stability profiles and weaker binding interactions, making M12 stand out in terms of both binding and overall receptor modulation.

The findings from this study demonstrated promising antidiabetic potential, particularly for compounds M12 and M7. Molecular docking showed that M12 and M7 had binding energies of -10.77 kcal/mol and -9.91 kcal/mol, respectively. These interactions were further supported by MD simulations, where the total energy values became significantly more negative—M12 at -48.25 kcal/mol and M7 at -40.13 kcal/mol, suggesting enhanced stability of the ligand–receptor complexes over time. These results were in line with previous in silico studies, such as the work by Olowosoke et al, who reported that natural compounds from *Citrullus lanatus* displayed favourable interactions with PPAR-γ and other diabetic targets, exceeding standard inhibitors in some cases (28). Similarly, in a follow-up study, several phytochemicals, particularly phytocassane A, outperformed standard drugs in docking and drug-likeness profiles against EZH2-PPAR-related targets, indicating their therapeutic potential for diabetes and related conditions (29). Dutta et al also identified stevioside as a stable PPAR-γ binder through molecular docking and dynamics simulations, with comparable binding energy values (30). Together, these findings supported the hypothesis that marine-derived compounds such as those from *H. tuna* might serve as viable candidates for PPAR-γ modulation in type 2 diabetes therapy, reinforcing the importance of natural product libraries in modern drug discovery efforts.

Conclusion

In silico evaluations of *H. tuna* natural compounds provided valuable insights into their potential as natural compounds as anti-T2DM agents. The molecular docking studies showed that M12 and M7 had binding energies of -10.77 kcal/mol and -9.91 kcal/mol, respectively. Furthermore, when MD simulations were conducted, the total energy components of M12 and M7 became more negative, with values of -48.25 and -40.13 kcal/mol, respectively. These findings suggested that M12, in particular, holds considerable promise as a candidate for further development in anti-T2DM therapies. Further research could involve experimental validation of these findings to confirm the natural compounds' efficacy and explore their potential in clinical settings.

Acknowledgement

The authors thank Bhakti Kencana University for laboratory access and funding of the 2024 DRPM Internal Research Grant, Universitas Bhakti Kencana, Indonesia.

Authors' contribution

Conceptualization: Aiyi Asnawi.
Data curation: Nina Nurafiani Putri and Hamidah Rahman.
Formal analysis: Nina Nurafiani Putri and Deden Indra Dinata.
Investigation: Nina Nurafiani Putri and Purnawan Pontana Putra.
Methodology: Aiyi Asnawi and Widhya Aligita.
Project administration: Ellin Febrina and Hamidah Rahman.
Resources: Ellin Febrina.
Software: Nina Nurafiani Putri and Aiyi Asnawi.
Supervision: Purnawan Pontana Putra.
Validation: Anne Yuliantini.
Visualization: Deden Indra Dinata.
Writing–original draft: Anne Yuliantini.
Writing–review & editing: Aiyi Asnawi and Widhya Aligita.

Conflict of interests

The authors have no competing interests that may be considered a potential threat to the objectivity of this research.

Ethical considerations

The authors of this study confirm that all ethical issues, such as copyright infringement, plagiarism, data creation, duplicate publication, and redundancies, have been considered and resolved. No animal or human subjects have been used in the present study.

Funding/Support

The study was funded by the 2024 internal research grant from DRPM Universitas Bhakti Kencana, under grant number 045/01/UBK/VII/2024.

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