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Potency of Bisindoles from Caulerpa racemosa in Handling Diabetes-Related Complications: In silico ADMET Properties and Molecular Docking Simulations

Muhammad Aswad[a, 1], Resky Nugraha[a], Risfah Yulianty[a], Ismail[a], Yayu Mulsiani Evary[a], Kasmiati[b], Zetryana Puteri Tachrim[c]

[a]Faculty of Pharmacy, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia
[b]Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia
[c]Research Centre for Chemistry, Indonesian Institute of Sciences, Kawasan Puspitek, Serpong, South Tangerang 15314, Banten, Indonesia

Abstract: Diabetes mellitus and its complications are among the primary causes of death and disability. Retinopathy, cardiovascular disease, and neuropathy develop progressively with prolonged hyperglycemia. Finding an effective and secure drug with fewer side effects to handle diabetes-related complications is necessary. Numerous scientists are launching new initiatives to investigate plant sources, which are known to contain a vast array of active agents. An edible marine algae, *Caulerpa racemosa*, was reported to have bioactivities including antidiabetes, anti-inflammatory and neuroprotective. Consequently, the current study was conducted to investigate bisindoles from *Caulerpa racemosa* using in silico method. Five bisindoles such as caulerpin, caulersin, racemosin A, racemosin B and racemosin C were selected to be anticipated their interaction binding mode and interaction energies toward protein targets associated with NF−κB such as TAK1 (7NTI), NIK (4IDV) and MMP−9 (4H3X) using AutoDock Vina® integrated with Chimera®, while their predicted ADMET were proceeded using web tool pkCSM®. The result indicated that all the compounds were predicted to interact molecularly with amino acids surrounding the binding site of protein targets while predicted pharmakokinetics and toxicity showed that most of the compounds meet the minimum standard parameters in ADMET properties. The findings suggested that bisindoles contained in *Caulerpa racemosa* might potentially to be used in treatment of diabetes-related complications.

Keywords: Caulerpa, ADMET, Bisindoles, Docking, Diabetes

1. Introduction

Diabetes is a metabolic disorder affecting a significant proportion of the global population. According the IDF Diabetes Atlas 2021, 537 million adults (20-79 years) have diabetes, or 1 in 10. This number is anticipated to reach 643 million by 2030 and 783 million by 2045 [1].

Diabetes can affect numerous organ systems and, over time, can result in severe complications. Both microvascular and macrovascular complications of diabetes can be identified. Damage to the nervous system (neuropathy), the kidneys (nephropathy), and the eyes (retinopathy) are examples of microvascular complications [2]. Hyperglycemia stimulates the formation of advanced glycation end products (AGEs) and an increase in reactive oxygen species (ROS). ROS and AGEs sequentially induce proinflammatory response and endothelial dysfunction by activating NF−κB signalling systems. Since NF−κB is a key role in the pathophysiology of diabetic vascular complications, inhibiting NF−κB may be an effective treatment [3].

The activation of NF−κB utilizes various kinases, such as transforming growth factor beta-activated (TGF-β) kinase 1 (TAK1) via the canonical NF−κB

[1] Corresponding Authors
e-mail: aswadfar@unhas.ac.id

pathway, while NF−κB inducing kinase (NIK) is the essential key in the non-canonical NF−κB pathway. Both canonical and non-canonical NF−κB pathways [4]. In particularly, TAK1 triggers the activation of TGF-β, which causes the diabetic kidney to accumulate extracellular matrix and develop fibrosis [5]. When NF-κB is activated, matrix metalloproteinase 9 (MMP-9) transcription is increased, which results in the development of diabetic retinopathy. In retinal capillary cells, MMP-9 is implicated in both angiogenesis and apoptosis [6–8].

There are numerous natural and synthetic NF−κB inhibitors, but their effects on diabetic complications are negligible [3]. Curcumin markedly decreases the diabetes-induced allodynia and hiperalgesia in rat [9] as well as diabetic retinopathy[10], while resveratrol demonstrated renoprotective effects in diabetic mouse via inhibiting Akt and JNK and reducing NF−κB activation [11].

Caulerpa racemosa (CR), an edible marine algae, has been shown to exhibit bioactivities that include antidiabetes as well as antioxidant [12–14], anti cancer [15] and anti-inflammation agent [16]. CR yields a wide variety of intriguing secondary metabolites, especially terpenoids and bisindole alkaloids [17] such as caulerpin, caulersin and racemosins [18–20]. In order to investigate the potency of bisindoles consisted in CR using in silico methods, the activity of bisindoles from CR was assessed in this work on prospective targets for diabetes-related complications (nephropathy, neuropathy and retinopathy) such as TAK1, NIK and MMP-9.

2. Computational Method

2.1 Preparation of ligands

Ligand structures consisting 5 bisindole compounds derived from *Caulerpa racemosa* [17,18,20] were constructed using section "Draw Structure" tool provided by https://pubchem.ncbi.nlm.nih.gov/, then generate the canonical SMILES structures (Tabel 1). The obtained structures were converted to PDB files by UCSF-Chimera® package.

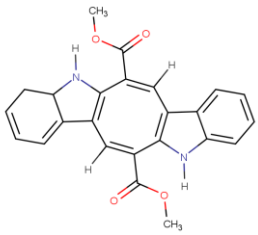
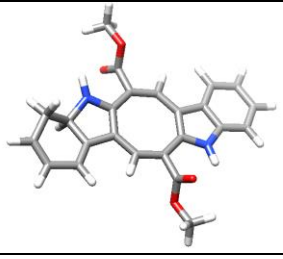
2.2 Molecular Docking Simulation

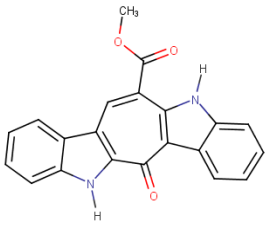
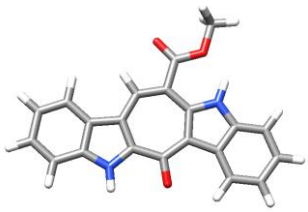
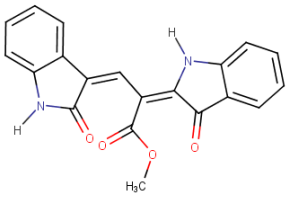
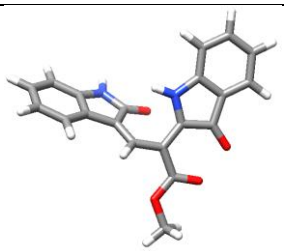
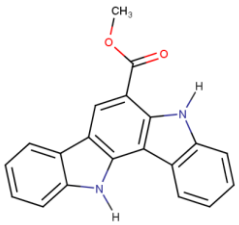
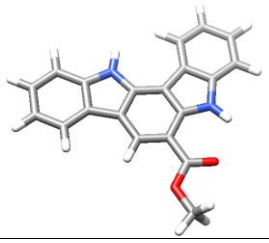
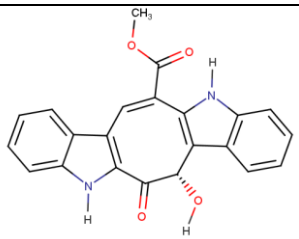
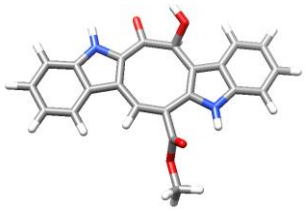
PDB structures of the compounds were minimized their structure using AM1-BCC algorithm. The crystal structure of protein targets derived from Protein Data Bank (https://www.rcsb.org/) such as TAK1 (PDB ID 7NTI)[21], NIK (PDB ID 1IDV)[22] MMP-9 homodimer protein in complex with an inhibitor (CC27) (PDB ID 4H3X) [23]were prepared to extract chain A from the dimer chain of the protein, subsequently hydrogen atom were added. Grids were set up for each protein target (Figure 1), which were TAK1 (7NTI) with grid box size 21x21x21 Å and grid center size 14.22, 14.32, 88.35 (xyz coordinate), NIK (4IDV) with grid box size 15x18x15 Å and grid center size -10.98, -21.32, -8.23 (xyz coordinate), and MMP-9 (4H3X) with grid box size 18x18x18 Å and grid center size 10.39, 7.33, 10.57 (xyz coordinate), respectively. Autodock Vina® associated with the UCSF-Chimera® package was utilized to conduct molecular docking, while visualization of the docking result was performed using Discovery Studio® software.

2.3 ADMET Properties calculations

ADMET properties were computed by inserting the canocinal SMILES data of the compounds into pkCSM® package [24] (http://biosig.unimelb.edu.au/pkcsm). The data obtained were collected and analyzed.

Tabel 1. List of bisindoles identified from *Caulerpa racemosa*

Compound	2D Structure	3D Structure
Caulerpin		

Caulersin		
Racemosin A		
Racemosin B		
Racemosin C		

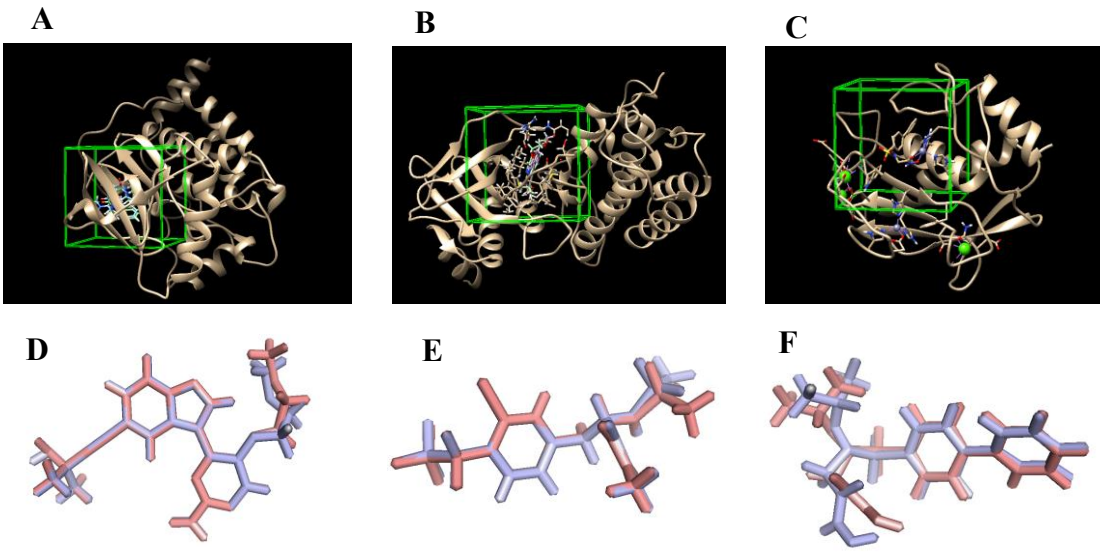


Figure 1. Preparation of target proteins with grid box (green) surrounding the binding pocket and overlay original ligan (pink) and re-docking ligand (violet). Protein TAK1(7NTI) (A); protein NIK (4IDV) (B); protein MMP-9 (4H3X) (C); overlay the original ligands (pink) and redocking ligands (purple) for TAK1 (D); NIK (E) and MMP-9 (F).

3. Results and discussion

3.1. Molecular docking analysis

Molecular docking simulations were selected to evaluate binding affinity of 5 bisindoles identified from *Caulerpa racemosa* toward target proteins. Transforming growth factor beta-activated kinase 1 (TAK1), NF−κB Inducing Kinase (NIK) and matrix Metalloprotease 9 (MMP-9) known as proteins involving in activating NF−κB were determined as proteins targets to treat diabetic vascular complications.

Redocking the original ligand into the protein's binding site served as the validation of molecular docking methods in order to ensure that they were reasonable. Figure 1 shows that the overlay of the original ligand's position with the re-docking ligands of TAK1, NIK, and MMP-9 has RMSD values of 0.005 Å, 0.074 Å, and 0.534 Å, respectively, less than 2.0 Å, indicating the molecular docking methods applied for each protein might be precise [25].

Tabel 2. Molecular docking result of ligands toward TAK1

No.	Ligand	Intermolecular energy (kcal/mol)	Hydrogen bond
1.	Original ligand	-7.8	Ser111, Ala107
2.	Caulerpin	**-8.2**	Ser111, Asp175
3.	Caulersin	**-9.0**	Ser111, Asp175
4.	Racemosin A	**-8.4**	Arg44, Val42
5.	Racemosin B	**-8.3**	Ser111, Asp175
6.	Racemosin C	**-9.1**	Ser111, Asp175, Arg44
7	Curcumin	-7.5	Asp175, Ser111, Ala107
8	Piperine	-7.0	Ser111
9	Resveratrol	-7.3	Arg44, Pro160, Glu105

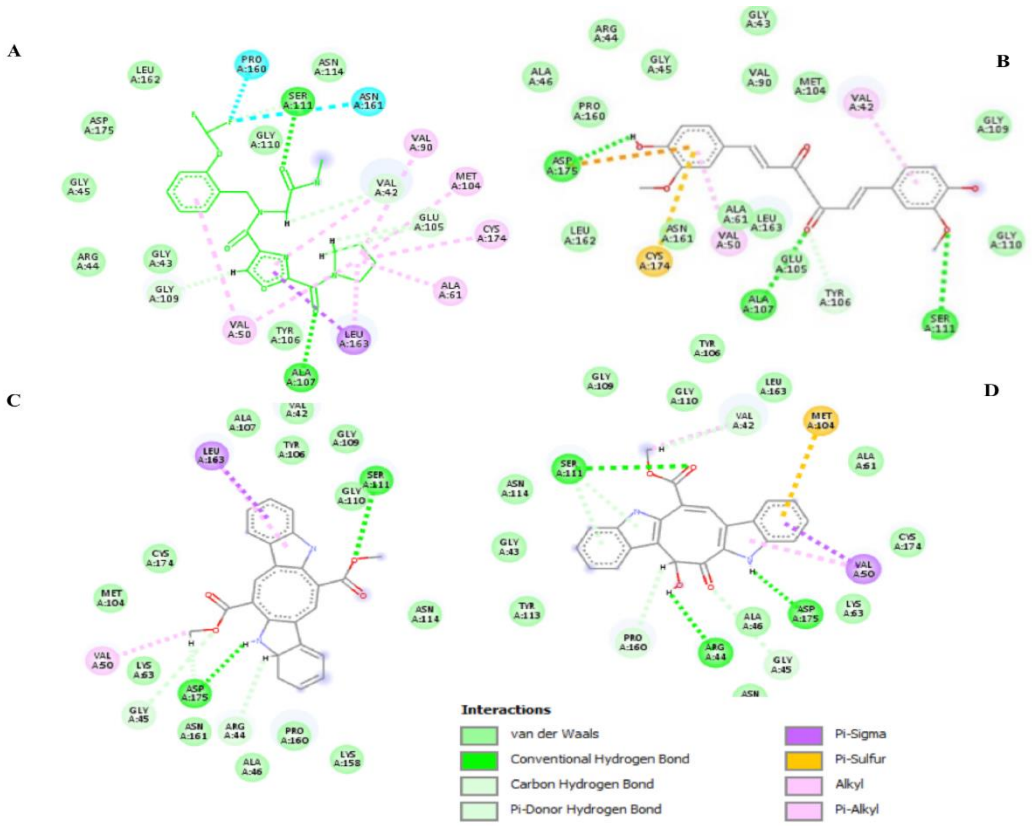


Figure 2. 2D ligan-target interactions at binding site of TAK1(7NTI), (A) original ligand, (B) curcumin, (C) caulerpin, (D) racemosin C

Redocking the original ligand into the protein's binding site served as the validation of molecular docking methods in order to ensure that they were reasonable. Figure 1 shows that the overlay of the original ligand's position with the re-docking ligands of TAK1, NIK, and MMP-9 has RMSD values of 0.005 Å, 0.074 Å, and 0.534 Å, respectively, less than 2.0 Å, indicating the molecular docking methods applied for each protein might be precise

All of bisindoles interacted tightly with amino acid residues sorrounding binding site of TAK1. Tabel 2 exhibits binding energy's values of bisindoles-target interaction (-8.2 to -9.1 kcal/mol) lower than original ligand (-7.8 kcal/mol) indicating that the compounds should attennuate TAK better than curcumin (-7.5 kcal/mol) and resveratrol (-7.0 kcal/mol), well-known anti-inflammatory agents reported to be involved in inhibition of NF−κB activation [3]. Figure 2 reveals all ligands except racemosin A bind Ser111, Ala107, or Asp175 that are key amino acid residues of TAK1 [21].

Racemosin B, caulersin and racemosin C were anticipated to suppress NIK activity by direct binding into the pocket of NIK. Tabel 3 displays ther value binding energy interaction of the compunds are -10.5, 10.7 and -11.0 kcal/mol respectively, lower than the original ligand (-9.6 kcal/mol). Since racemosin B exhibited significantly neuroprotective effect [18], that might be associated with inhibition to NIK, a crucial kinase in non-canonical pathway of NF−κB activation.

Binding interactios of racemosin B into the binding pocket of NIK as described in figure 3 are dominated by hydrophobic interactions such a phi-alkyl, phi-sigma and phi-sulfur while the only hidrogen bond was created between its carbonyl group with Ser476.

MMP-9 plays important role in diabetic retinopathy. Suppression of this enzyme activity might be an alternative way to treat diabetes-associated retinopathy [6]. As displayed in table 4 as well as figure 4, all of bisindoles bind directly ion Zn^{2+} located in active catalytic region of the enzyme. Racemosin A exhibits the lowest intercation energy (-10.0) that not significantly different with piperine (-10.1), an alkaloid that reported to diminish MMP-9 activity [26].

Tabel 3. Molecular docking result of ligands toward NIK

No.	Ligand	Intermolecular energy (kcal/mol)	Hydrogen bond
1.	Original ligand	-9.6	Glu440, Glu470, Leu472, Ser476, Phe535,
2.	Caulerpin	-9.3	Ser410, Ser476, Gln479,Asp534
3.	Caulersin	**-10.7**	Ser410
4.	Racemosin A	-8.9	Ser476, Cys533
5.	Racemosin B	**-11.0**	Ser476
6.	Racemosin C	**-10.5**	Ser410, Ser476, Asn520, Asp534, Gln479
7.	Curcumin	-8.3	Ser476, Leu472, Asn520, Gln479
8.	Piperine	-8.4	Gln479
9.	Resveratrol	-8.0	Leu472, Glu470, Asp534, Asn520, Ser410

Table 4. Molecular docking result of ligands toward MMP-9

No.	Ligand	Intermolecular energy (kcal/mol)	Hidrogen Bond	Interaction with Zn
1.	Original ligand	-8.8	Glu122, Ala84, Leu83	Metal acceptor
2.	Caulerpin	-8.0	Glu122	Metal acceptor
3.	Caulersin	-8.6	Glu122, Tyr143	Metal acceptor
4.	Racemosin A	**-10.0**	Glu122, Leu83, Tyr140	Pi-cation
5.	Racemosin B	-8.1	Glu122	Metal acceptor
6.	Racemosin C	-8.4	Glu122, Ala84, Tyr143	Metal acceptor
7.	Curcumin	**-8.9**	Leu83, Ala84, Tyr143	-
8.	Piperine	**-10.1**	-	Metal acceptor
9.	Resveratrol	**-8.9**	Leu83, Ala84, Met142, Arg144	-

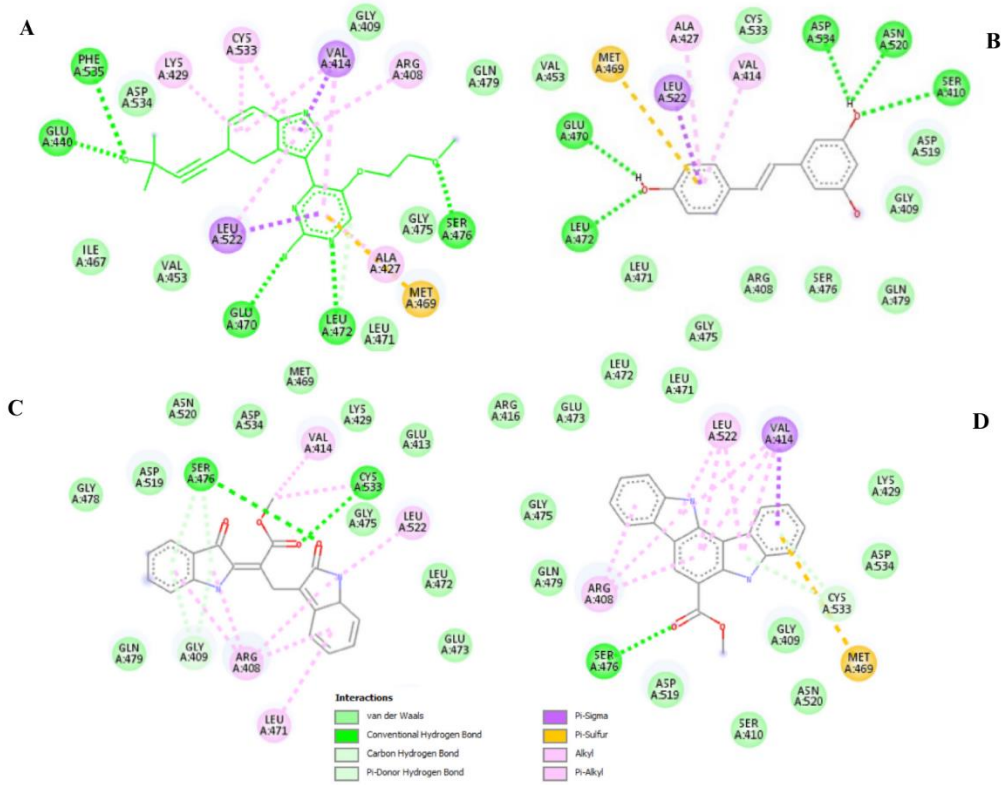


Figure 3. 2D ligan-target interactions at binding site of NIK (4IDV), (A) original ligand, (B) reveratrol, (C) racemosin A, (D) racemosin B

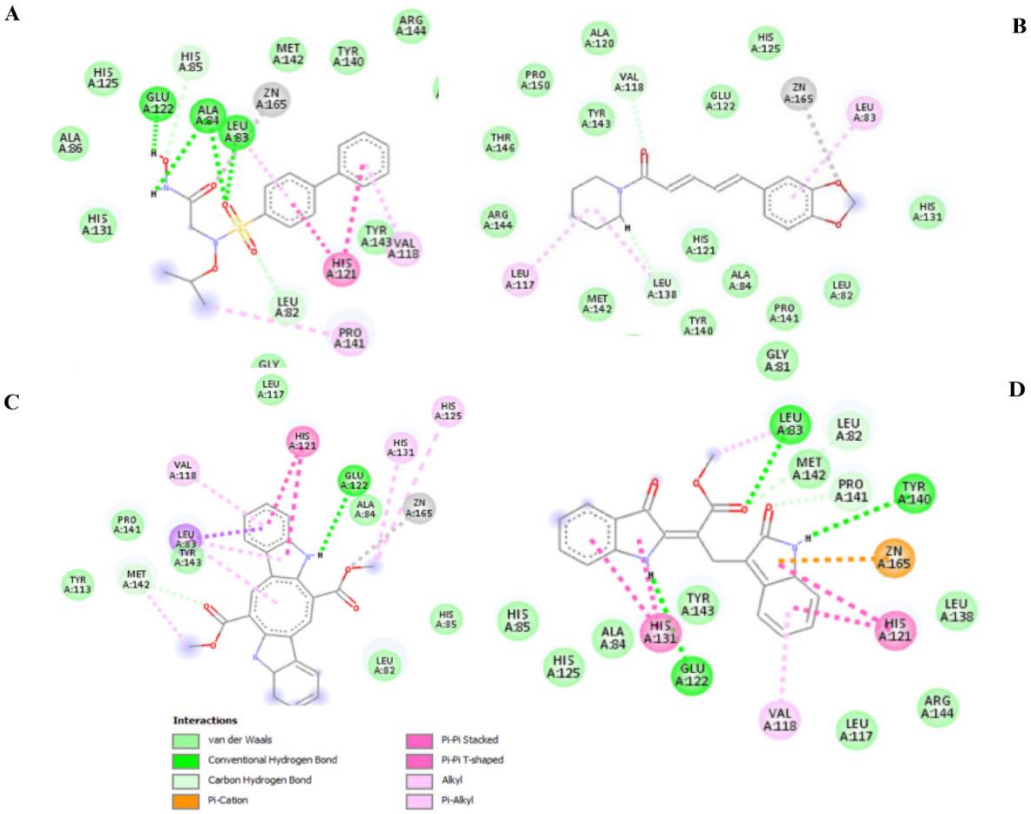


Figure 4. 2D ligan-target interactions at binding site of MMP-9 (4H3X), (A) original ligand, (B) piperin, (C) caulerpin, (D) racemosin A

3.2 ADMET Properties

The result of the ADMET properties calculation displayed in Table 5 indicates that all of the compounds were anticipated to have excellent absorbtion in human instestine (more than 95%); however, racemosin C was predicted to have poor Caco-2 permeability. All of the compounds are considered P-gp substrate as well as the possibility to be P-gp I and II inhibitors.

The distribution parameters are revealed by volume distribution (VDss), fraction unbound (Fu) and BBB-permebility. Caulerpin's VDss is considered high with logVDss = 0.514 (logVDss > 0.45) as well as its Fu while Racemosin B is predicted low with logVDss = -0.881 (loggVDss < -0.15) [24]. All of the compounds are considered to enter BBB slightly (poorly distributre to the brain if logBBB < -1), subsequenly they are anticipated to penetrate the CNS (logPS> -2)

All ligands are metabolized by CYP3A4 however, they might not metabolize by CYP2D6. On the other hand they are anticipated to be inhibitors of CYP3A4, CYP2C19 and CYP2C9. All of the compounds are predicted hepatotoxicity without skin sentitation effect. However, caulerpin and racemosin B domonstrated high acute toxicity based on minnow toxicity prediction with logLC50 < -0.3.

Table 5. Predicted ADMET properties of bisindoles using pkCSM

	Properties	Ligand				
		Caulerpin	**Caulersin**	**Racemosin A**	**Racemosin B**	**Racemosin C**
Absorbtion	Caco2 permebility	1.073	0.763	0.868	1.249	0.32
	Intestinal absorbtion	95.117	96.39	95.156	95.097	100
	Skin permebility	-3.23	-2.737	-3.012	-2.735	-2.735
	P-gp substrate	Yes	Yes	Yes	Yes	Yes
	P-gp I inhibitor	Yes	No	Yes	Yes	Yes
	P-gp II inhibitor	Yes	Yes	No	Yes	Yes
Distribution	VDss	0.514	-0.141	-0.11	-0.881	0.365
	Fraction unbound	0.15	0.108	0.014	0.065	0.069
	BBB permeability	-0.027	-0.015	0.103	0.232	-0.696
	CNS permeability	-2.01	-1.62	-2.14	-1.443	-1.868
Metabolism	CYP2D6 substrate	No	No	No	No	No
	CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes
	CYP1A2 inhibitor	No	Yes	No	Yes	Yes
	CYP2C19 inhibitor	Yes	Yes	Yes	Yes	Yes
	CYP2C9 inhibitor	No	Yes	Yes	Yes	Yes
	CYP2D6 inhibitor	No	No	No	No	No
	CYP3A4 inhibitor	Yes	Yes	Yes	Yes	Yes
Excretion	Total Clearance	0.719	0.757	0.122	0.885	0.431
	Renal OCT2 substrate	No	No	No	No	No
Toxicity	AMES toxicity	No	Yes	No	Yes	No
	HERG I inhibitor	No	No	No	No	No
	LD50 (rat, oral, mol/kg)	2.518	2.661	2.585	2.336	2.643
	Hepatotoxicity	Yes	Yes	No	Yes	Yes
	Skin sensitation	No	No	No	No	No
	Minnow toxicity (logLC50)	-0.365	0.176	0.464	-0.939	1.534

4. Conclusions

The study accurately predicted the effectiveness of the bisindoles found in *Caulerpa racemosa* using molecular docking simulation. The results suggest that these compounds could be used to treat complications related to diabetes, such as neuropathy, nephropathy, and retinopathy, by blocking NF-κB-related proteins like TAK1, NIK,

and MMP-9. Racemosin C was anticipated to be the most potent compound to inhibit TAK1, followed by Racemosin B for NIK and Racemosin A for MMP-9. Predicted ADMET properties indicated the compounds met some parameters of pharmacokinetics and toxicity.

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