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Screening Emodin Derivatives as DPP-4 Inhibitor Candidates: *In Silico* and *In Vitro* Assessment

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disease distinguished by disrupted glucose metabolism, causing elevated blood sugar levels. One of the latest therapeutic strategies involves inhibiting dipeptidyl peptidase-4 (DPP-4) to regulate glucose metabolism. Emodin, a bioactive compound, has shown potential as a DPP-4 inhibitor, but its efficacy requires further research. This study aims to identify and assess emodin and its derivatives as potential DPP-4 inhibitors through a comprehensive in silico and in vitro analysis. Molecular docking analysis revealed that 3-ρ-toluoyl emodin (ρTE) had the lowest binding energy (-111.4 kcal/mol) among the tested compounds. Furthermore, in vitro testing showed consistent results in silico, indicating that ρTE had significant inhibitory activity with an IC_{50} value of 1.37 μM. Pharmacokinetic and physicochemical evaluations confirmed ρTE's potential as a safe antidiabetic drug candidate. The research findings indicate that ρTE holds potential as a promising drug candidate for further development.

Keywords: Antidiabetic, DPP-4 inhibitor, Emodin, Diabetes mellitus

INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by a deficiency in insulin production or function, leading to elevated blood sugar levels (Okur et al., 2017). According to the International Diabetes Federation (IDF), approximately 537 million adults (20-79 years) were diagnosed with diabetes in 2021, and this number is expected to increase to 643 million by 2030 and 783 million by 2045 (IDF Diabetes Atlas, 2021). Current antidiabetic therapies often show limited efficacy and side effects, prompting the search for alternative treatment options. An innovative method to manage glucose levels is to inhibit DPP-4, an enzyme that degrades incretin hormones, which are essential for regulating blood glucose levels (Q. Wang et al., 2018). Another strategy involves searching for novel medicinal sources from natural products. Various herbal plants possess antidiabetic activity, attributed to the availability of bioactive compounds, including emodin (Mulyati & Seulina Panjaitan, 2021).

Emodin (Fig. 1), a natural anthraquinone, has shown potential as a DPP-4 inhibitor, with previous in silico studies reporting an inhibition value of 1.41 μM and a binding energy of -7.98 kcal/mol at the DPP-4 active site (GDP ID: 4A5S) (Davella et al., 2022). Furthermore, emodin has been reported as a DPP-4 inhibitor in silico, with a Gibbs energy value of -5.06, using glibenclamide as a positive control (Ravindran & Dorairaj, 2016). This compound has also been reported to inhibit the DPP-4 enzyme with an IC50 value of 5.76 μM (Davella et al., 2022) in an in vitro assessment. In in vivo assessments, emodin has also been reported to inhibit the DPP-4 enzyme in Balb/C and ob/ob (-/-) mice at a dose of 30 mg/kg (Z.Wang et al., 2017). However, research on emodin derivatives remains limited. This study examined the capacity of emodin derivatives to inhibit the DPP-4 through a dual approach involving in silico molecular docking and in vitro enzyme inhibition analysis.

In this study, modifications were performed in silico as an initial analysis to modify proteins and develop new drugs. This analysis can reduce trial and error in laboratory testing and save costs and time for new drug development (Sumaryada et al., 2017). In vitro analysis was conducted on compounds previously analyzed in silico to establish a correlation between the two analysis results (Natsir et al., 2024). This study introduces a novel approach to developing antidiabetic drugs by modifying emodin compounds to enhance their activity. With limited research on emodin derivatives targeting DPP-4 protein (PDB ID: 5T4B),

this research offers new insights and potential breakthroughs in diabetes treatment.

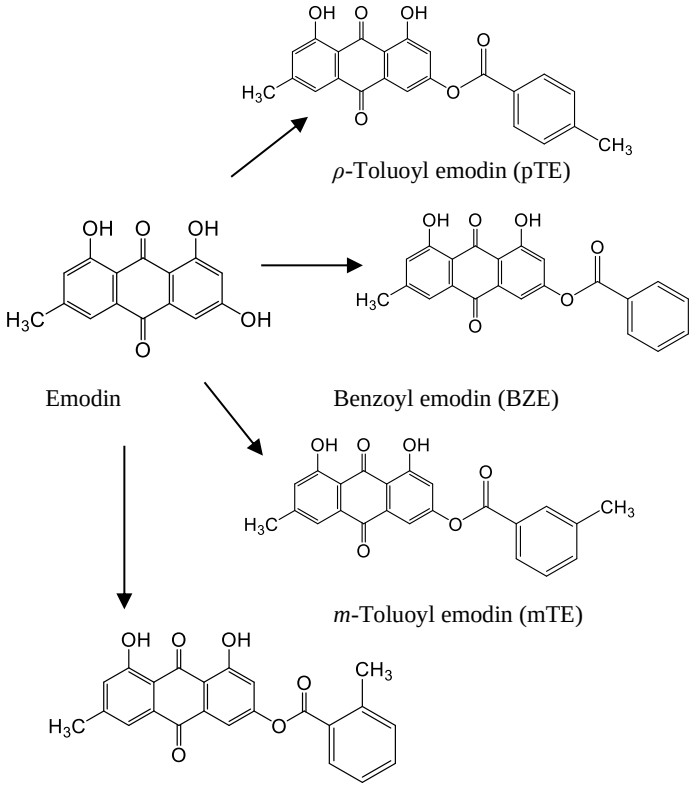


Figure 1. Emodin and derivative structures

METHODOLOGY

Materials and Instrumentals

Molecular docking was performed using Molegro Virtual Docker 6.0 (2013), and three-dimensional molecular structures were designed using ChemDraw 2020. Pharmacokinetic and physicochemical analyses were conducted using the Molinspiration and SwissADME software. In vitro enzyme inhibition assays were performed using a DPP-4 inhibitor screening kit (Sigma-Aldrich).

Receptor and Ligand Preparation

The DPP-4 crystal structure (PDB ID: 5T4B) was accessed from the Protein Data Bank. Protein preparation involves the removal of water molecules and native ligands to optimize ligand binding. To prepare for docking simulations, the emodin derivatives were transformed into 3D conformers and optimized through energy minimization. Sitagliptin was used as the reference ligand.

Molecular Docking

Molegro Virtual Docker 6.0 (MVD) was used to dock molecules to the active site of the DPP-4 enzyme. Molecular docking is a method used to predict the binding mode and free energy between a ligand and a receptor. The ligand-receptor interaction was analyzed based on the docking scores, which measure the complex's stability. Molecular docking also allows for predicting the specific interactions between ligands and amino acid residues within the protein's active site (Hanum et al., 2021). Molecular docking allows the determination of the grid box value and coordinates (x: -4.35, y: 60.33, z: 38.04) that define the search space for ligand-receptor interactions, enabling accurate identification of protein active sites and enhancing docking method accuracy (Kim et al., 2018). The Root Mean Square Deviation (RMSD) value was employed as a parameter to assess the interaction between the protein and ligand before and after the docking process. The RMSD value is considered good if ≤ 2 Å (Zubair et al., 2020). This stage analyzes several parameters, such as the rerank score and hydrogen bonding. Visualization and analysis of the results will be performed using Biovia Discovery Studio 2024 (Sulfahri et al., 2019).

Analysis of Drug-likeness and Predicted ADMET Profiles

Drug-likeness scores were predicted using Molinspiration (https://www.molinspiration.com/) and SwissADME (http://www. swiss). ADMET refers to absorption, distribution, metabolism, excretion, and toxicity, which describe the processes of drug absorption, distribution, metabolism, excretion, and toxicity. The ADMET profile is valuable for predicting drug candidates' pharmacological and toxicological properties, particularly in the preclinical stage. In silico models have been developed to improve ADMET predictions. Using these models has significantly contributed to drug optimization and avoided late-stage failures, which is crucial because such failures result in substantial unproductive investments of time and money (Domínguez-Villa et al., 2021).

In Vitro Enzyme Inhibition Assay

DPP-4 inhibition was assessed following the MAK203 protocol (Shaikh et al., 2022). Each well contained 25 µL of sample, 49 µL of DPP-4 assay buffer, and 1 µL DPP-4 enzyme. The fluorescence intensity was measured at λex = 360 nm and λem = 460 nm using a microplate reader.

RESULTS AND DISCUSSION

Receptor and Ligand Preparation Optimization

The preparation and optimization processes were performed to obtain the optimal protein and ligand structures, which were the most stable and suitable for their conditions. Preparation of the DPP-4 protein involved separation from its native ligand using BIOVIA Discovery Studio 2024 Client. As a result of this separation, two distinct structures were obtained: a protein molecule without the native ligand and a distinct native ligand structure. This separation aimed to generate a binding site on the DPP-4 protein, providing a pocket for test compounds to interact with.

Emodin and its derivatives, as test ligands, underwent energy minimization and geometry optimization of their structures using the molecular mechanics (MM2) method 1000 times using the Chem3D Professional program. A similar process was applied to the native ligand bound to the DPP-4 protein. The purpose of this stage was to evaluate the modified ligand structure using Lipinski's Rule of Five. This test aimed to ensure that the ligand met the standards of an effective drug candidate by the Lipinski RO5 principle. The criteria to be met included a molecular weight <500 g/mol, low lipophilicity (LogP <5), hydrogen bond donors <5, and <10 hydrogen bond acceptors (Ino Ischak et al., 2023).

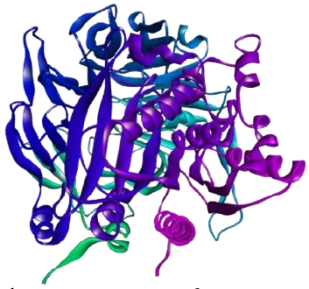


Figure 1. 3D Structure of DPP-4 protein after preparation and optimization

Molecular Docking Studies

Molecular docking analysis was performed to investigate the potential binding interactions between the compound and the enzyme's active site (Danova et al., 2023). The validation process ensured that the molecular docking method met the necessary criteria for reliable analysis. The Root Mean Square Deviation (RMSD) value provided a quantitative measure of the protein-ligand interaction, allowing for the analysis of binding mode changes occurring before and after the docking process. The RMSD value is considered good if ≤ 2 Å (Zubair et al., 2020). The RMSD value was 0.391 Å, indicating that the docking method used was valid.

In addition to protein and ligand validation, the success of molecular docking methods is also indicated by the rerank score value of a ligand. The lower the rerank score, the stronger the binding between the compound and the target enzyme (Mavillapalli et al., 2017). According to the docking results, ρTE had the highest binding affinity, indicated by the lowest binding energy (-111.4 kcal/mol), as shown in Table 1, indicating a strong interaction with the active site of DPP-4. Compared to emodin (-79.186 kcal/mol) and sitagliptin (-75.943 kcal/mol), ρTE showed a higher binding affinity. The presence of hydrogen bonds with key active site residues further supports its potential as a potent inhibitor. Therefore, it can be said that ρTE has potential as an antidiabetic drug.

Table 1. Ligand docking result

	Ligand	Rerank Score	H Bond
Native Ligand	75N	-128.88	-5.312
Reference Ligand	Sitagliptin	-75.943	-5.043
Test Ligand	Emodin	-79.186	-7.616
	ρ-Toluoyl emodin	-111.4	-6.780
	m-Toluoyl emodin	-110.071	-4.572
	o-Toluoyl Emodin	-109.26	-4.485
	Benzoyl emodin	-103.431	-2.625

Furthermore, the docking process elucidates the interaction activity between amino acids and ligands with their respective proteins, thereby facilitating a deeper understanding of the mechanisms of action of ligands and proteins.

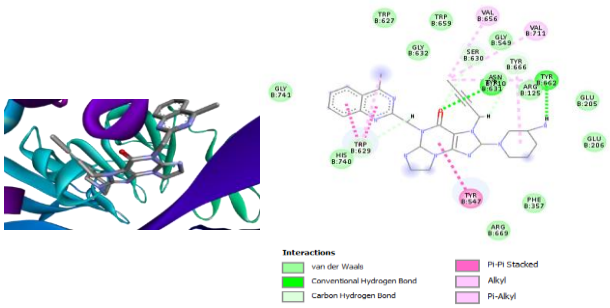
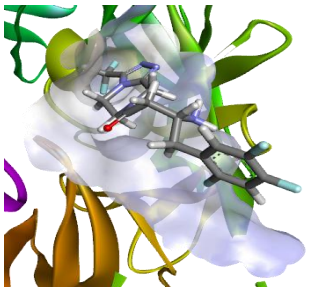
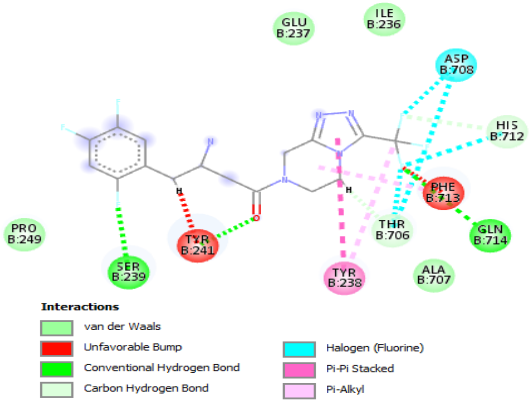
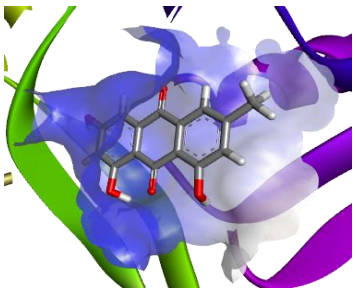
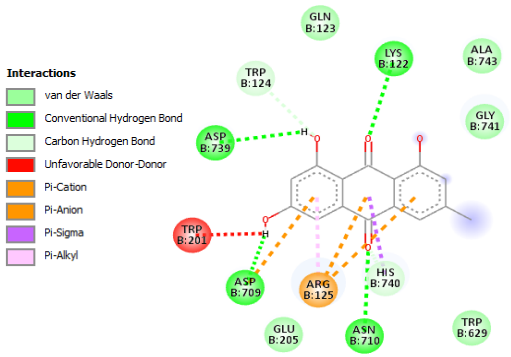
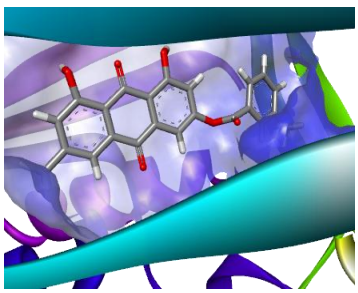
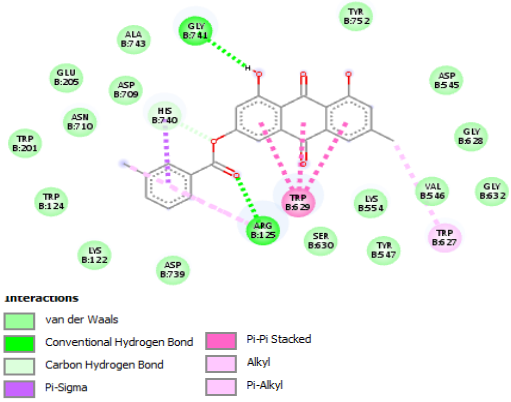


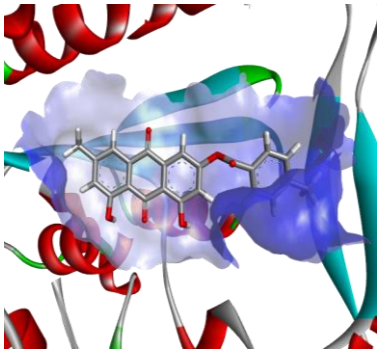
Figure 2. Interaction of native ligand on DPP-4 protein

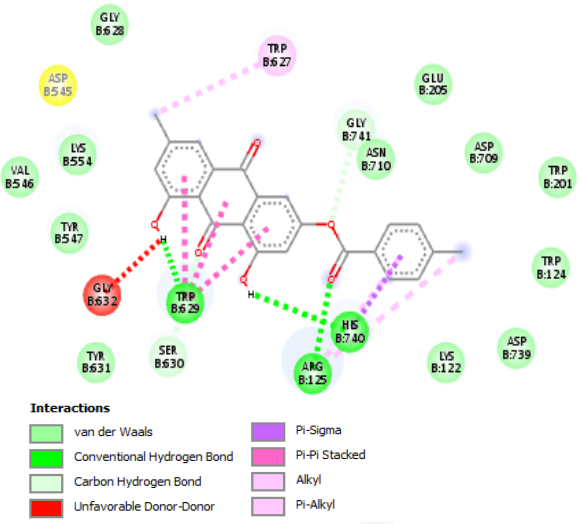
This knowledge can be exploited to design novel ligands with enhanced efficacy (Reynaldi et al., 2023). As shown in Table 2, the interactions between the amino acids and the test ligands were characterized by hydrogen bonding. Hydrogen bonding is a critical factor in augmenting the affinity between amino acids and ligands, modulating their orientation, enhancing stability, influencing enzyme activity, and increasing the specificity of amino acid-ligand interactions, thus minimizing the likelihood of non-specific interactions (Zhao et al., 2021). Docking analysis reveals that emodin derivatives engage in strong hydrophobic interactions with the receptor. Specifically, ρTE forms hydrogen bonds with Arg125, Trp629, and His740, whereas *m*TE interacts with Arg125 and Gly741 via hydrogen bonds, and *o*TE exhibits hydrogen bonding with Arg125, Trp629, and Asn710.

Table 2. Hydrophobicity and amino acid interaction with ligands

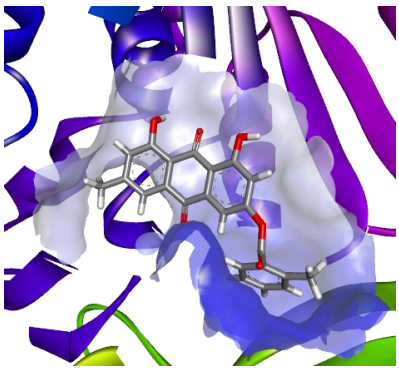
Ligand	Hydrophobicity	Interaction
Sitagliptin		
Emodin		
m-Toluyl emodin		

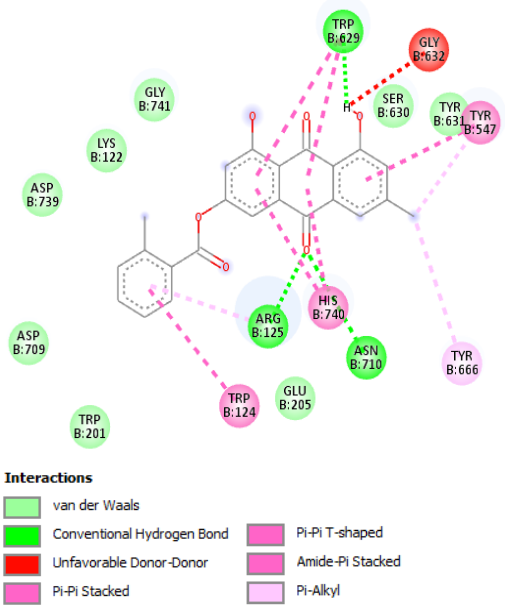
ρ-Toluyl emodin



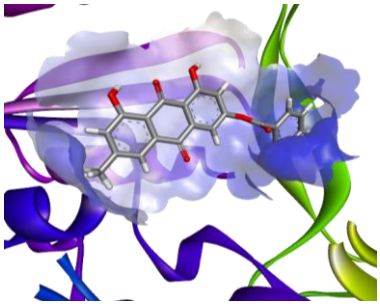


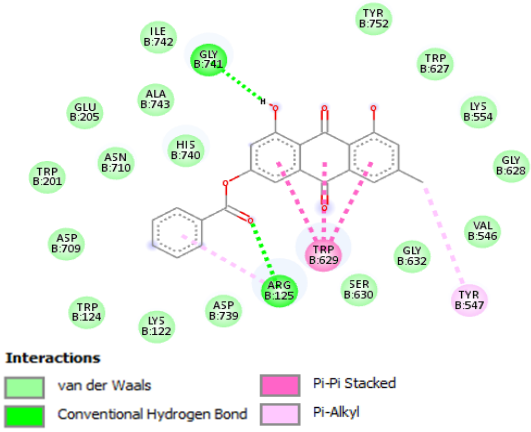
o-Toluyl emodin





Benzoyl emodin





ADMET profiles, and Pharmacokinetics

The ADMET profile prediction and pharmacokinetics results confirmed that emodin derivatives met Lipinski's Rule of Five (Table 3), suggesting favourable bioavailability. ADMET predictions indicated no significant hepatotoxicity, hERG inhibition, or AMES toxicity. The Lipinski RO5 rule was used to determine a compound's oral bioavailability by considering the compound's solubility and permeability in the gastrointestinal tract (Masyita et al., 2024). The emodin derivatives, ρTE, *m*TE, *o*TE, and BZE, comply with ADMET parameters, exhibiting favourable pharmacokinetic properties, including molecular weight <500 g/mol, Log P <5, ≤5 hydrogen bond donors, ≤10 hydrogen bond acceptors, and PSA <140 Å, thereby qualifying as potential drug candidates (Table 3). Pharmacokinetic prediction aims to optimize a drug's performance, including absorption, distribution, metabolism, and effective and safe dose design, to achieve the desired therapeutic effect (Ivanov et al., 2018). Based on these parameters, the emodin derivatives can also be considered safe as drug candidates, as shown in the data presented in Table 4.

Table 3. The ADMET profiles of ligand

Ligand	MW (gr/mol)	Log P	PSA (Å)	RB	HBA	HBD
75N	481.55	2.9	112.18	4	6	1
Sitagliptin	407.31	2.06	77.04	6	10	1
Emodin	270.24	3.01	94.83	0	5	3
m-Toluoyl emodin	388.37	5.8	100.90	3	6	2
ρ-Toluoyl emodin	388.37	5.8	100.90	3	6	2
o-Toluoyl emodin	388.37	5.8	100.90	3	6	2
Benzoyl emodin	374.34	5.36	100.90	3	6	2

Table 4. Pharmacokinetics prediction of ligand

Ligand	AMES toxicity	hERG inhibitor	Hepatotoxicity	Skin Sensitization	GI Absorption	BBB Permeant
75N	No	No	Yes	No	High	No
Sitagliptin	No	No	Yes	No	High	Yes
Emodin	No	No	No	No	High	No
m-Toluoyl emodin	No	No	No	No	High	No
ρ-Toluoyl emodin	No	No	No	No	High	No
o-Toluoyl emodin	No	No	No	No	High	No
Benzoyl emodin	No	No	No	No	High	No

DPP-4 Inhibition Assay

The results of molecular docking, ADMET, and pharmacokinetic analyses showed that ρTE exhibited the highest inhibitory activity (IC_{50} = 1.37 μM), surpassing emodin (IC_{50} = 2.34 μM) and other derivatives, as shown in Figure 3. These results align with the in-silico findings, reinforcing the potential of ρTE as an antidiabetic agent.

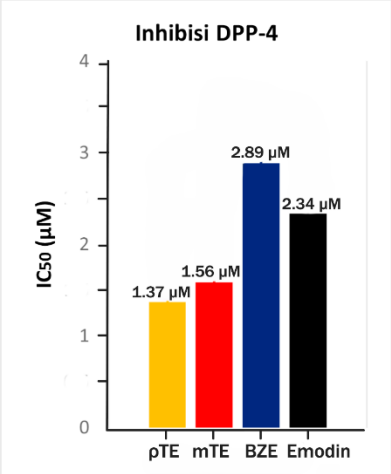


Figure 3. DPP-4 inhibitory potential of emodin derivatives

CONCLUSION

The in silico and in vitro methods used in this study produced consistent and corresponding results, indicating that emodin derivatives have potential inhibitory activity against DPP-4 protein, suggesting that emodin derivatives could be considered candidate antidiabetic drugs. This study shows that ρ-toluoyl emodin (ρTE) has better potential for inhibiting DPP-4 protein, exhibiting superior binding affinity and enzymatic inhibition relative to emodin and sitagliptin. In vitro results further confirmed the inhibitory potential of ρTE, making it a strong candidate. More in vivo research using animal models is needed to solidify this strategy's effectiveness.

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Declaration of Interest

The authors declare no competing financial conflicts of interest.

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