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Anti-diabetic Activity of Phloretin Against Maltase-glucoamylase Using Docking, Pharmacokinetics and Pharmacophore Studies

Miah Roney[1,2], Mohd Fadhlizil Fasihi Mohd Aluwi[1,2,*]

[1]*Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang Darul Makmur, Malaysia.*
[2]*Centre for Bio-Aromatic Research, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang Darul Makmur, Malaysia.*

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ABSTRACT

A metabolic condition known as diabetes mellitus is characterized by hyperglycemia brought on by either inadequate insulin production, resistance to insulin action, or both. The anti-diabetic medications that are now on the market either impact how much glucose is absorbed and excreted or how much insulin is secreted without affecting insulin resistance. This study used in-silico methods to assess the efficacy of phloretin as anti-diabetic drug against maltase-glucoamylase. Phloretin displayed strong binding to the maltase-glucoamylase protein with good docking scores (-7.4 kcal/mol), as opposed to co-crystallized ligand (-6.2 kcal/mol). In terms of physicochemical qualities, drug-likeness, and medicinal chemistry properties, Phloretin also performed best. The results of molecular docking and data acquired from pharmacokinetic characteristics of phloretin may be used further for the creation of newer maltase-glucoamylases with potential anti-diabetic properties and improved pharmacokinetic profiles.

1. Introduction

Diabetes mellitus (DM) is a long-term, progressive condition marked by problems with protein, lipid, and glucose metabolism. Approximately 403 million diabetics were diagnosed globally in 2019, and 700 million were expected to have the disease by 2045 [1]. Diabetes is a global threat and a matter for healthcare reform since diabetics have a greater risk of death and morbidity than do healthy persons [2]. The anti-diabetic medications now on the market either alter glucose absorption and excretion or boost insulin secretion without affecting insulin resistance [3].

There are several established anti-diabetic targets, but maltase-glucoamylase, which breaks down alpha-glucosidic linkages in carbohydrates, is one of the key targets for insulin resistance and is essential for regulating postprandial blood glucose levels [2]. By rupturing the internal alpha-1,4 linkages in the amylopectin and amylase structures of starch, salivary and pancreatic alpha-amylases break down the disaccharides into branched and linear dextrin chains, imitating the breakdown of the disaccharides. Prior to being taken into the circulation, dextrins are further converted by maltase-glucoamylase into glucose molecules [4]. Maltase-glucoamylase can be used to prevent type 2 diabetes by blocking or reducing the enzymatic process that transforms dextrin to glucose molecules, which delays or inhibits the synthesis of glucose in diabetic or pre-diabetic people [5].

Flavonoids are a group of naturally occurring phenolic chemicals that are produced in plants as bioactive secondary metabolites and are in charge of giving food its flavor, color, and pharmacological effects. Strong antioxidants, they shield plants from harmful environmental factors [6]. According to research flavonoids have immunomodulatory [7], antiviral [8], antimicrobial [9], anti-inflammatory [7], anticancer [10], and anti-diabetic [11, 12] properties. One of the main categories of naturally occurring flavonoid chemicals is

∗ Corresponding author. Mohd Aluwi M.F.F., e-mail: fasihi@ump.edu.my
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the chalcones [6]. Chalcones and their derivatives play a significant role in medicinal chemistry because they have a variety of pharmacological properties [13], including anti-inflammatory [14], antimicrobial [15], antiviral [16], antioxidant [15], cytotoxic [17], antitumor [18], and anti-diabetic [19] properties.

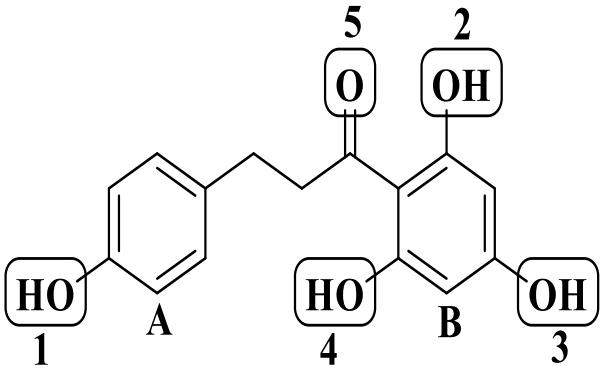

Fig 1. Possible site to synthesis Phloretin derivatives

Dihydrochalcones (also known as 1,3-diaryl-2-propen-1-ones) are phenolic compounds having a diphenylpropan (C6-C3-C6) flavonoid skeleton and no heterocyclic C ring. In fact, the majority of these secondary metabolites in plants are precursors of flavonoids. The interactions with another functional group are carried out by phloretin, a crystalline phenolic ketone comprising two aromatic phenol rings (ring A and ring B), hydroxyl groups (1-4), and a carbonyl group (5) (Fig 1) [20]. A wide variety of pharmacological activities are also controlled by these functional groups. The carbonyl and OH groups of ring A contribute to the antioxidant activity of 20,60-dihydroxyacetophenone, the antioxidant pharmacophore of phloretin, whereas one hydroxyl moiety is substituted by some sugar, which reduces the activity relative to phloretin.

According to Behzad et al., [20], more than 300 plant families have produced roughly 200 dihydrochalcones thus far. Phloretin is mostly obtained from pears (*Pyrus communis*) [21] and apple trees (*Malus domestica* L.) [22, 23]. A double bond location on the three carbon atoms that bridge the homocycle rings A and B, as well as the heterocyclic C ring, are absent from this phenylpropanoid. Phloretin is a particularly adaptable molecule that can effectively connect with biological macromolecules thanks to the former. Phloretin has a wide range of pharmacological effects, including those of an anti-diabetic [24], cardioprotective [25], hepatoprotective [26], anti-inflammatory [27], antioxidant [28], immunosuppressive [29], and antimicrobial [30].

We speculate that phloretin may interact and serve as anti-diabetic drugs based on the anti-diabetic effects of flavonoids and chalcones. In this work, we used *in-silico* methods to examine the effects on diabetics of Phloretin.

2. Results and Discussion

2.1. Active site prediction

The proper putative binding pocket in the target molecules is discovered at this crucial stage of the docking process. Even though the protein has several binding sites, only one of them can serve as a potential ligand binding pocket [31]. This binding pocket identification identified almost five drug-grade pockets as suitable ligand binding sites. The site values were measured afterwards, and a single docking point was chosen (Fig 2). The volume of the active site was 1042 and the center was x= -0.2, y= 3.6 and z= -32.8 as well as the size was x= 18, y= 18 and z= 17. Furthermore, the active residues of this site were Lys776, Glu510, Phe522, Thr775, Ala780, Ala509, Phe535, Thr778, Val779, Lys513, Arg520, Ile523, Ala285, His645, Ala536, Ala537, Ala512, Ser288, Pro287, Lys534, Gly533, Ser521, Ala291, Asp777, Leu286, Val506 and Met567.

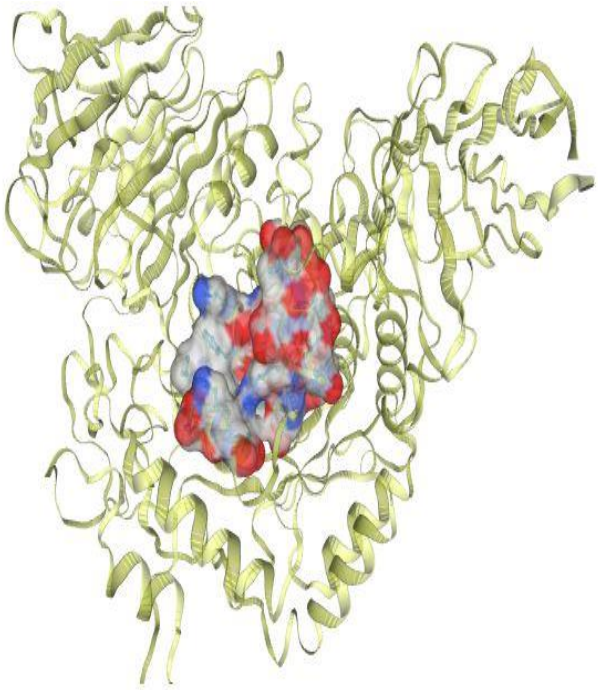

Fig 2. Active site prediction of maltase-glucoamylase protein (PDB ID: 3L4Y)

2.2. Docking

The molecular interactions and mechanisms of binding of ligands with the target receptor are revealed by molecular docking [3]. Using the online docking program CB-Dock, the ligands were subjected to an in-silico molecular docking study at the active ligand binding site of the maltase-glucoamylase protein (PDB: 3L4Y) to determine the likely molecular mechanism underlying the anti-diabetic effect. With the target protein, Phloretin showed excellent docking scores (Table 1). After automatically finding the binding sites, calculating the centre and size, and tailoring the docking box size for the query ligands, the protein-ligand blind docking method known as CB-Dock employs AutoDock Vina to carry out the molecular docking [32]. In this investigation, we employed blind docking to examine how the phloretin interact to the active site or allosteric site. From the active

site prediction and docking results, phloretin bind at the active site of maltase-glucoamylase protein.

Table 1. Binding analysis of Phloretin and Co-crystallized ligand with maltase-glucoamylase protein (PDB ID: 3L4Y)

Compound Name	Vian Score	Bound Amino Acids
Co-crystallized ligand	-6.2	Thr775, Leu286, Asp777, Lys534, Pro284, Arg283, His645, His115 (H-B), Ala509, Lys534 (C-H), Arg520, His645 (ionic)
Phloretin	-7.4	Pro287, Thr775, Leu286, Arg520, Pro284 (H-B), Leu286, Ala285, Ala509, Lys534 (C-H), Arg520 (ionic), His645, Lys776 (π-cation)

The hydrophobic pocket of the target receptor was found to properly match the chemicals. Fig 2 depicts the binding modes of phloretin and co-crystallized ligand. The docking energies and their distributions for interactions such hydrogen bonds, hydrophobic bonds, ionic bonds, π-π bonds, and π-cation interactions are shown in Table 1. The docked conformation of the phloretin and co-crystallized ligand in the binding sites of the 3L4Y protein and their 2D interactions are depicted in Fig 3a and 3b. The total energy scores of phloretin with the target protein was anticipated using docking calculations. Since the phloretin-3L4Y complex has the best inhibitory impact, with a total energy score of -7.4 kcal/mol, compared to the co-crystallized ligand-3L4Y (-6.2 kcal/mol).

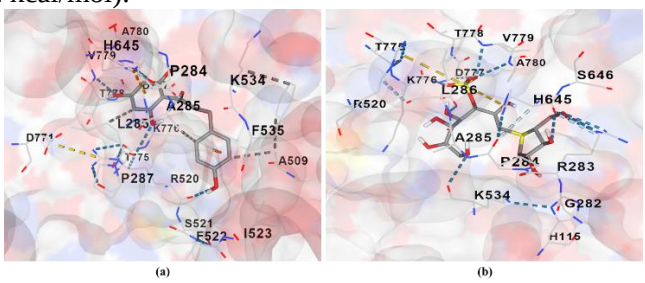

Fig 3. Docked pose of (a) Phloretin and (b) Co-crystallized ligand with maltase-glucoamylase protein (PDB ID: 3L4Y)

A thorough study of the data revealed that the ligands and surrounding residues interacted in a variety of ways, including hydrogen bonds, hydrophobic bonds, ionic bonds, π-π bonds, and π-cation interactions. The docked position of phloretin revealed two hydrophobic contacts with Ala509 and Lys534, two ionic connections with Arg520 and His645 residues, and five hydrogen bond interactions with amino acids of Thr775, Leu286, Asp777, Lys534, Pro284, Arg283, and His645. We were able to pinpoint specific amino acid residues, including Thr775, Leu286, Asp777, Lys534, Pro284, Arg283, His645, and His115, as hydrogen, hydrophobic, and ionic interactions within the binding pocket of 3L4Y using docking analysis with the co-crystallized ligand. Our in-silico docking studies show that phloretin binds to the active site of 3L4Y, suggesting that phloretin may has anti-diabetic properties against the maltase-glucoamylase protein.

2.3. Pharmacokinetics Study

Table 2. Pharmacokinetics profiles of Phloretin and Co-crystallized ligand.

	Physicochemical characteristics					Drug-likeness						Medicinal Chemistry			
ompoundName	**W**	**PSA**	**A**	**D**	**R**	**ipinski**	**hose**	**eber**	**gan**	**uegge**	**ioavailability Score**	**AINS**	**rank**	**eadlikeness**	**ynthetic accessibility**
eferenceValue	**500**	**140**	**5**	**10**	**0-130**	**ess**	**ess**	**ess**	**ess**	**ess**	**--**			**es**	**--**
o-crystal	24.44g/mol	61.95Å²		2	5.17	o; 2 violation	o; 1 violation	o; 1 violation	o; 1 violation	o; 4 violation	.17		alerts: charged_o	o; 2 violations	.72

			s: HA, HD	:WLOGP<-0.4	:TPSA>140	:TPSA>131.6	s: XLOGP3<-2, TPSA>150, H-acc>10, H-don>5		xygen_sulfur, sulfonic_acid_2, sulphate	:MW>350, Rotors>7				
horetin	74.27g/mol	79Å²		4.02	es	es	es	es	es	.55	alert	es	.88		

A drug candidate's appropriateness is not always assured by an inhibitor's antagonistic reaction to an enzyme or a protein receptor. As a result, drug-likeness analysis has been essential in the process of discovering new drugs since it helps in determining whether to test potential inhibitors against biological systems or not [33]. The primary physicochemical factors investigated in these experiments are molecular weight, topological polar surface area (TPSA), hydrogen bond donors and acceptors (HBD and HBA), and molar refractivity.

The results show that phloretin has the best results for the physicochemical properties, drug-likeness, and medicinal chemistry scores. Due to the molecular weights of phloretin and co-crystallized ligand were 274.27 g/mol and 424.44 g/mol, respectively—and TPSAs of 97.99 Å^2, which was higher than 75 Å^2 but lower than 140 Å^2. On the other side, the TPSA of the co-crystallized ligand was 261.95 Å^2, which was hiher than the reference value (Table 1). Phloretin was appeared to be suitable for use as medication. Phloretin also includes four hydrogen bond acceptors (nON) and five hydrogen bond donors (nONH), both of which fall inside the permitted range. Phloretin adhered to the medicinal chemistry rule and drug-likeness properties. The co-crystallized ligand did not meet the additional requirements for being chosen as an oral medication. Phloretin was determined to be the best choice for a neutral molecule to function as an effective oral medication based on the research results.

2.4. Common Pharmacophore Features

Pharmacophore techniques have advanced over the past century and are now one of the most important tools in the drug development process. There have been several ligand-based and structure-based methods developed for improved pharmacophore modelling. These techniques have been successfully and widely used to lead optimisation, de-novo design, and virtual screening [34]. In the lack of a macromolecular target structure, ligand-based pharmacophore modelling has emerged as a crucial computational method [31]. Despite these achievements, pharmacophore techniques have not yet operated to their full potential, particularly considering the need to lower the high total costs currently connected with drug research and development [33]. Phloretin was found to have the hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), and aromatic ring (R) (Fig 3). The common pharmacophore reveals that phloretin was composed of two acquired acceptors (A1 and A2), six obtained donors (D1 and D6), one obtained hydrophobics (H1), and one obtained aromatic ring (R1) (Fig 4).

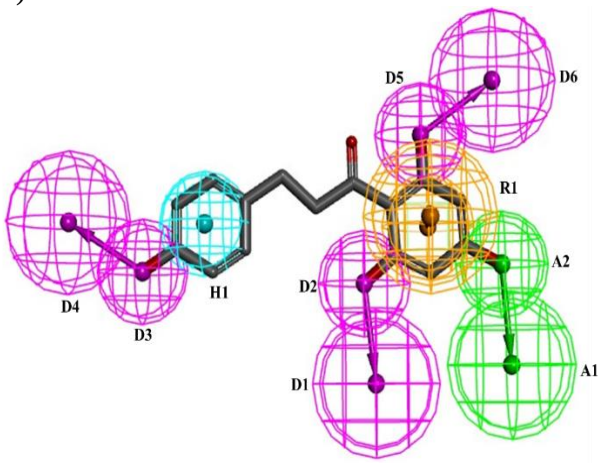

Fig 4. Pharmacophore hypothesis of Phloretin. A denotes hydrogen bond acceptor in green color, D denotes hydrogen bond donor in rose, H denotes hydrophobic in blue color and R denotes aromatic rings in orange color.

3. Experimental

3.1. Target protein selection and Active site prediction

The maltase-glucoamylase crystal structure (PDB ID: 3L4Y), which has a sequence length of 875 and a resolution of 1.8 Å, was selected from the literature and obtained from the PDB database [35].

There are a multitude of binding sites on the protein, but without identifying the active site, not all of them could be identified as suitable docking sites. After a thorough examination, a suitable site was determined using the DrugRep online tool for the docking of the phytochemicals with this protein [36].

3.2. Ligand selection and preparation

Based on the anti-diabetic activity of flavonoids and chalcones as well as the anti-diabetic activity of phloretin, we selected phloretin as ligand to dock with maltase-glucoamylase (PDB ID: 3L4Y) protein. The reference compound was the co-crystallized ligand. Phloretin and co-crystallized ligand were constructed using the ChemSketch and saved in .mol format.

3.3. Docking

A previously described method [37] was utilised to estimate the docking analysis utilising the online docking programme CB-Dock. Investigated were the interactions between the ligands and the maltase-glucoamylase protein. Prior to docking, a PBD file for the receptor and a.mol file for the ligands were input into the CB-Dock programme. Several top cavities were automatically chosen throughout this approach and used for further investigation (cavity sorting), with molecular docking carried out at each one. The docked position with the highest AutoDock Vina score and biggest cavity was chosen for further investigation after the binding modalities were examined.

3.4. Pharmacokinetics Study

The Swiss ADME online server was utilized for the physicochemical properties, drug-likeness, and medicinal chemistry research [33]. To create physicochemical properties, drug-likeness, and medicinal chemistry specifications, phloretin and co-crystallized ligand were imported into the program through SMILES.

3.5. Common Pharmacophore Features

The Discovery studio 3.1 software was employed to identify the common pharmacophore characteristics of phloretin. The maximum number of features that could be created was 6, the lowest distance between features was set at 2, the minimum distance between features of the same kind was set at 4, and donors were used as vectors.

4. Conclusion

Development of new molecules with potential biological action and few to no side effects is urgently needed to treat diabetes effectively. Phloretin was effectively docked onto the active site of the maltase-glucoamylase protein, which is responsible for insulin sensitization, in the current work. The fitness score of the proposed compound was determined using the CB-Dock program. Maltase-glucoamylase's interactions with 3L4Y varied in terms of hydrogen bonds, hydrophobic interactions, π-cation interactions, and ionic interactions; however, docking scores support the idea that up-regulating maltase-glucoamylase may have significant anti-diabetic properties. The findings of this study suggest that may serve as the starting points for the creation of novel anti-diabetic drugs, and they have the potential to be used in the future to successfully treat diabetes. To address the possible biological activity of phloretin with maltase-glucoamylase, more molecular biology research on cell culture and/or animal studies will be helpful.

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