Journal of Engineering and Technology

ISSN 2180-3811 eISSN 2289-814X https://jet.utem.edu.my/jet/index

VIRTUAL SCREENING BY IN SILICO MOLECULAR DOCKING AND PHARMACOKINETIC OF CHALCONE HYBRID AS α-GLUCOSIDASE INHIBITOR

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Article history:
Received Date: 5 July 2024
Revised Date: 1 October 2024
Accepted Date: 21 November 2024

Keywords: Anti-diabetes, α-Glucosidase Inhibitor, Chalcone,

Abstract— Diabetes, particularly type 2, is increasing in prevalence every year and has emerged as the third-most significant global health issue. One of the critical approaches to targeting enzymes that regulate carbohydrate metabolism is the α-glucosidase enzyme. Inhibiting this enzyme can reduce glucose absorption in the blood by causing the carbohydrates to break down. Commercially available drugs usually have unwanted side effects; hence, the development of novel drugs is a must. This current study aims to develop anti-diabetic

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Docking, SwissADME	drugs using a computational approach to screen out the best compounds (1–9). We performed in *silico* molecular docking using Auto Dock 4.0 and visualized the results using PyMOL and Discovery Studio. The study found binding energies (BE) that were greater than or equal to acarbose (-8.08 kcal/mol) and between -6.65 and -8.70 kcal/mol. The drug-like properties, pharmacokinetics, toxicity profile, and drug score were performed using the SwissADME, AdmetSAR, and Molsoft programs. Compounds 1–9 obeyed the Lipinski Rule of Five, and most of the compounds had drug-like properties and were non-toxic. Besides, they have promising interactions with α-glucosidase enzyme. Hence, they have the potential to develop into potent anti-diabetic drugs with lesser toxicity.

I. Introduction

According to the International Diabetes Federation (IDF 2024), there are currently 540 million people worldwide who have diabetes, and this number is expected to continue increasing. Projections suggest that by 2045, almost 783 million individuals between the ages of 20 and 79, which is equivalent to 1 in 8 persons, would be affected with diabetes (IDF 2024). Diabetes type 2 affects a majority of the population, with over 90% of individuals being affected [1]. Possible factors contributing to this issue include urbanisation, an ageing population, a decrease in healthy habits, and a loss in physical activity (IDF 2024). Nevertheless, it is possible to contemplate preventative actions by means of timely diagnosis, appropriate medical attention, and a well-balanced

lifestyle [2]. Diabetes type 2 is a prevalent condition that is deemed serious due to its potential to induce problems in essential organs [3]. Diabetes type 2 is characterised by impaired insulin activity or insulin resistance, leading to an increase in glucose levels known as postprandial hyperglycemia (PPHG) [4]. PPHG has long-term consequences such as retinopathy, nephropathy, and cardiovascular problems [5].

Chalcone is a natural compound exists naturally in fruits and plants, considered privileged in medicinal chemistry because of versatile biological properties [6-8] and convenient synthesis. Chalcone derivatives had been reported have an excellent α-glucosidase inhibitory activity like chalcone oxime (1.61 μM) [9] and hydroxylchalcone (12.5 μM) [10]. Besides, chalcone have tolerance in human body [7] and itself can reduce toxicity. For example, chalcone incorporation with BODIPY molecular probes reduces its cytotoxicity values on normal cell with 102.21 μM [11]. While hybridization of two bioactive compounds can increase the pharmacological efficacy [12]. Sulfonyl moiety has been found to possess potential anti-diabetic action [13]. Adding a sulfonyl moiety to the essential oil of *Origanum vulgare* L. increases bioactivity against α-glucosidase fifty times better than acarbose [14]. Therefore, we will design the chalcone hybrid with sulfonyl to create potent drugs as shown in Figure 1.

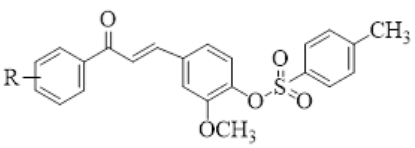


Figure 1: Proposed structure of chalcone arylsulfonate ester hybrid

A common technique and widely used since the early 1980s in the design of the drugs is molecular docking, a computer technique that envisages the attachment of the target drug into site of amino acid residue. The docking approach allows us to elucidate binding modes of compounds in in the binding site of target proteins and their interactions at the atomic level [15]. Prediction of the drugs conformation and

evaluation of the binding affinity are essential in the docking process [15]. They use a scoring algorithm to compare binding energies to identify the optimal docking solution among various orientations [16]. While researchers have previously invested significant time and money in conducting in *vivo* and in *vitro* investigations during the drug development process [17-18]. Previous studies showed that the results of scoring function were compatible with biological testing [19]. Therefore, we can use docking results to screen the behaviour of the compounds and eliminate unwanted ones. While molecular docking has not yielded ample data, it can serve as a first stage in the development of potential pharmaceuticals. The objective of the project is to examine the interaction between chalcone hybrids and α-glucosidase by molecular docking. Additionally, the pharmacokinetic and toxicity profiles of these hybrids will be assessed using SwissADME and AdmetsAR prior to doing further biological testing. Considering this, there are high chances that the designed drugs will be successfully become potent drugs.

II. Materials and Methods

A. Molecular docking

In this study, we examined eighty chalcone hybrids and selected only nine compounds, which are chalconetoluenesulfonylester (CTSE) derivatives. Ligands were prepared by minimizing their geometry and energy to create 3D coordinates, which were then saved as PDB using the Avogrado programs. Next, select the torsion option in Auto Dock 4.2, identify the roots and determine the number of torsions before saving the file as a PDBQT file. The 3D crystallographic structure of protein with ligand (PDB ID: 5NN8) was downloaded from the RSCB protein data bank [12]. Protein was dehydrated and adding polar hydrogen bonding using Autodock 4.2. After saving the grid preparation of the protein as PDBQT, enter the coordinates of ligands in the grid box (X: 13.389, Y: -38.216, Z: 95.021) for docking purposes,

and save as gpf format. The docking process was performed by choosing a protein rigid file name (PDBQT) and ligand (PDBQT), using the Lamarkian genetic algorithm parameter, and saving in dpf format [18]. Then using the dpf format, execute Auto Dock 4.0 from ADT. The interaction between protein and ligand was visualised using PyMol and Discovery Studio Visualiser.

B. In-*Silico* Prediction of Drug-Likeness, Pharmacokinetic and Toxicity

The drug-like properties, pharmacokinetics and toxicity profiles of target drugs were elucidated by using online web resources: SwissADME (http://www.swissadme.ch) and admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) [20], [21], [22]. The drug-like score was evaluated using Molsoft program (Molsoft L.L.C.: Drug-Likeness and molecular property prediction).

III. Results and Discussion

A. Molecular docking

Nine distinct ligands of chalconetoulenesulfonyleester (CTSE) were subjected to comparative docking analysis to determine their binding affinity with human lysosomal acid α-glucosidase (GAA) (PDB ID: 5NN8) [23]. To begin, the co-crystallized ligand acarbose was re-docked into the active site of the α-glucosidase enzyme to validate the docking parameter [16]. The validation is considered successful when the ligand-protein complex accurately reproduces the crystallographic binding orientation with a root mean square deviation (RMSD) value of the docking less than 2.0 Å which was 1.83 Å as illustrated in Figure 2.

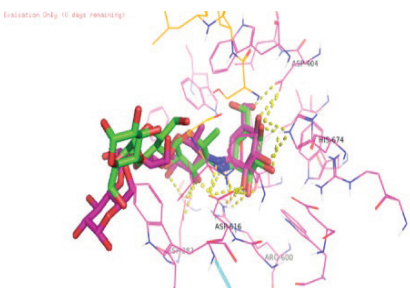


Figure 2: Superimposed structure of crystallographic ligand (purple) and docked acarbose (green) in the active site of the GAA (PDB ID: 5NN8)

After validation, the designed compounds CTSE (1-9) were docked to the active site of the enzyme. The findings demonstrated notable and moderate binding energies (BE) compared to acarbose (-8.08 kcal/mol), with values in the range of -6.65 to -8.70 kcal/mol, as illustrated in Table 1 (appendix). The lowest BE is significant because it has greater possibility of binding with enzyme and tends to have higher inhibitory activity.

The compound that has the highest BE was 2-methyl-CTSE (2) (-8.70 kcal/mol), with two H-bonding with amino acid residues ASP282 and ARG600. Figure 3 (appendix) showed that it no longer interacts with ASP616, ASP404, or HIS674. Instead, it establishes a new hydrophobic interaction with ASP518 (π-anion), PHE649 (π-π stacked), LEU678 (alkyl), MET519 (sulfur-X), TRP516 (π-alkyl), HIS674 (π-alkyl) and TRP481(π-anion). This could potentially account for the compound's higher binding energy (BE) compared to acarbose. ARG600 amino acid residues form hydrogen bonding with the oxygen moiety of the derivative. While ASP282 established a weak hydrogen bond between the OH moiety of amino acid residue and the carbon atom of methoxy group.

The second active molecule, 3-methyl-CTSE (3), had a binding energy (BE) of -8.25 kcal/mol. This compound formed two hydrogen bonds with GUY651 and SER676, as well as three weak carbon hydrogen bonds with ASP616, LEU650, and LEU678. The hydrophobic interaction involves LEU678 (π-alkyl and alkyl), LEU677 (alkyl), LEU650 (π-alkyl), TRP376 (alkyl), ASP616 (π-anion), and TRP481 (π-sulfur and π-π T-shaped). The docking conformation with the third highest activity was 4-methyl-CTSE (4), with a binding energy of -7.96 kcal/mol. The molecule formed two hydrogen bonds with LEU677 and LEU678, which is the oxygen atom of the sulfonate ester group. Hydrophobic interactions occur between TRP376 (π-π stacked), LEU678 (π-alkyl), TRP481 (alkyl), ASP518 (π-anion),

TRP516 (π-alkyl), PHE649 (alkyl), HIS674 (alkyl), LEU650 (π-alkyl), and TRP618 (alkyl). Compound 2 exhibits a slightly greater binding energy (BE) compared to compounds 3 and 4, most likely because of the impact of the methyl group's position on the affinity and conformations that can fit into the active site of the enzyme.

A comparison of BE with compound 1 without R groups indicates that the presence of electron-donating groups (EDG) lowered the BEs, whereas the presence of electron-withdrawing groups (EWG) like nitro and fluoro increased the BEs. This study suggests that EDG enhances electron density and readily donates to the enzyme receptor to establish a beneficial interaction. Furthermore, all designed compounds 1 to 9 showed better binding energy compared to the chalcone scaffold alone (10) due to increased interactions with enzyme receptors. Figure 3 (appendix) illustrated binding modes of active compounds 2-4.

B. In-*Silico* Prediction of Drug-likeness, Pharmacokinetic and Toxicity

According to the swissADME evaluation depicted in Table 2 (appendix), it suggests that all the designed compounds obey the Lipinski rule of 5 without any violations. They showed molecular weight (MW) < 500, H-bond donor (HBD) < 5, H-bond acceptor (HBA) < 10, log p < 5, TPSA < 140, and rotatable bond (RB) < 10 [24]. Their drug score using Molsoft program is in the range of -0.74 to -0.18 as illustrated in Figure 4 (appendix), which all lie in the range of the likeness of drug and has potential to develop as a therapeutic drug.

Pharmacokinetic and toxicity screening were illustrated in Table 3 (appendix) and Table 4 (appendix). Six of the nine compounds 1-4 and 8-9, exhibited high gastrointestinal (G1) absorption, which is a good indicator of good oral bioavailability. While all the compounds cannot penetrate blood-brain permeability (BBB), it is encouraging that the enlisted

compounds have the potential to be diabetic medications and will not affect the brain. Compounds 1-9 have potential become P-gp inhibitors, which can enhance the efficiency of the drug delivery into the body by preventing active efflux across membranes.

Cytochrome P450 (CYP450) isoenzymes are the primary precursors of drug metabolism and play a crucial role in eliminating drugs through metabolic biotransformation. The five primary isoforms include CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Blocking the activity of these specific enzymes results in the buildup of medicines and the possibility of harmful effects. Based on the information provided in Table 3 (appendix), it can be concluded that compounds 1–9 do not possess the ability to block all forms of CYP450. Consequently, these compounds are unlikely to accumulate in the body and cause toxicity. Toxicity screening revealed that most of the compounds, except for 6 and 7, had possible non-mutagenic properties. Nevertheless, all the chemicals exhibited carcinogenicity in a binary context but demonstrated non-carcinogenicity in a trinary context. The predicted LD_{50} in rats for acute toxicity fall within the range of 1.67–2.26 mol/kg.

IV. Conclusion

The binding free energies of each residue towards their respective active site were computed. Remarkably, the study revealed that the intermolecular hydrogen bonding inside the active site had significantly lesser impacts on the binding free energies. In contrast, the binding free energy resulting from hydrophobic contacts exhibited dominant interaction at the active site of the α-glucosidase enzyme. Based on the dock score, the three compounds most likely to be active were 2-4.

These compounds, consisting of electron-donating groups (EDG), have the potential to increase electron density and donate electrons to the receptor, thereby promoting stability. While the varying positions of R

groups at benzene ring of chalcone can change the conformations that fit into the binding site pocket. According to the admet analysis, most of the compounds meet the criteria for potential development as therapeutic drugs with minimal toxicity. Based on these findings, new compounds will be synthesized with enhance ability to bind to target molecules, improve their pharmacokinetic properties, and minimize their toxicity. These compounds will next undergo biological testing. While the varying positions of R

groups at benzene ring of chalcone can change the conformations that fit into the binding site pocket. According to the admet analysis, most of the compounds meet the criteria for potential development as therapeutic drugs with minimal toxicity. Based on these findings, new compounds will be synthesized with enhance ability to bind to target molecules, improve their pharmacokinetic properties, and minimize their toxicity. These compounds will next undergo biological testing.

V. Appendix

Table 1: Interaction of compounds 1-10 and α-glucosidase inhibitor (acarbose) in the active site of GAA

Compound	Amino acid Residues		Binding Energy (kcal/mol)
	Polar interaction (H-bonding)	Hydrophobic interaction	
1	ALA281, ARG600	LEU283, PHE525, ASP282, MET519, TRP481, PHE649, TRP376	-7.88
2	ASP282, ARG600	ASP518, PHE649, LEU678, MET519, TRP516, HIS674, TRP481	-8.70
3	GUY651, SER676	LEU678, LEU677, LEU650, TRP376, ASP616, TRP481	-8.25
4	LEU677, LEU678	TRP376, LEU678, TRP481, ASP518,	-7.96

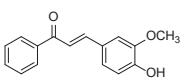
5	ASP616, ARG281	TRP516, PHE649, HIS674, LEU650, TRP618 ALA284, ALA555, LEU650, PHE649, TRP481, MET519, TRP376, TRP516, ILE441	-6.55
6	ARG600, LEU677, LEU678, GLY651,	TRP516, ILE441, HIS674, PHE649, TRP376, ASP518, TRP481,	-7.16
7	GUY651, SER676, ALA284	ASP282, ASP616, ALA555, LEU650, LEU678,	-7.79
8	ASP616	ASP616, MET519, LEU650, TRP481, ASP518, PHE649, TRP516, ILE441, HIS674	-7.43
9	LEU677	LEU650, LEU678, TRP376. TRP481, ASP616	-7.31
10 	ARG600	ASP404, ASP616, MET519, ILE441, LEU405	-5.62
Acarbose	ASP616, ASP282, ARG600, ASP404, HIS674	-	-8.08

Table 2: Physicochemical properties and Druglikeness

Compound	Physicochemical properties						Drug-likeness	Bioavailability score
	MW	RB	HBA	HBD	TPSA	iLogP	Lipinski	
1	408.47	7	5	0	78.05	3.63	Yes	0.55
2	422.49	7	5	0	78.05	3.84	Yes	0.55
3	422.49	7	5	0	78.05	3.89	Yes	0.55
4	422.49	7	5	0	78.05	4.01	Yes	0.55
5	453.46	8	7	0	123.87	3.10	Yes	0.55
6	453.46	8	7	0	123.87	3.35	Yes	0.55
7	453.46	8	7	0	123.87	3.40	Yes	0.55
8	426.46	7	6	0	78.05	3.73	Yes	0.55
9	426.46	7	6	0	78.05	3.70	Yes	0.55

Table 3: Pharmacokinetic prediction using SwissADME

Compound	GI absorption	BBB permeant	P-gp	CYP450 inhibition				
				1A2	2C19	2C9	2D6	3A4
1	High	No	Yes	No	Yes	Yes	No	No
2	High	No	Yes	No	Yes	Yes	No	No
3	High	No	Yes	No	Yes	Yes	No	No
4	High	No	Yes	No	Yes	Yes	No	No
5	Low	No	Yes	No	Yes	Yes	No	No
6	Low	No	Yes	No	Yes	Yes	No	No
7	Low	No	Yes	No	Yes	Yes	No	No
8	High	No	Yes	No	Yes	Yes	No	No
9	High	No	Yes	No	Yes	Yes	No	No

Table 4: Toxicity prediction of compounds 1-9

Compound	Mutagenicity	Carcinogenicity		Acute oral toxicity
	Ames test	Binary	Trinary	(mol/kg)
1				1.83
2				2.01
3				1.85
4				2.02
5				1.77
6				1.67
7				1.68
8				2.09
9				2.26

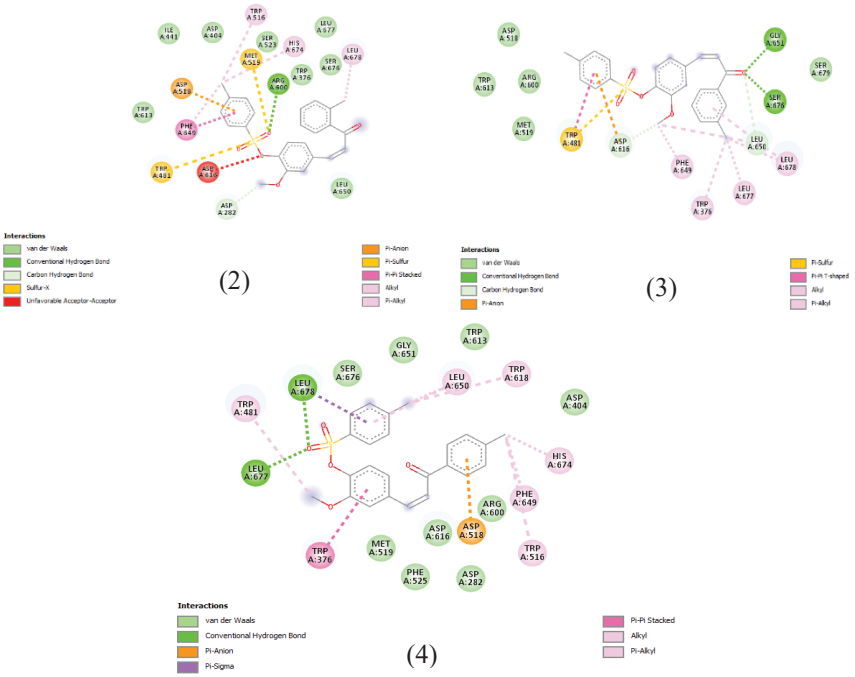


Figure 3: Binding modes of active compounds 2-4 using Auto Dock 4.0 molecular docking

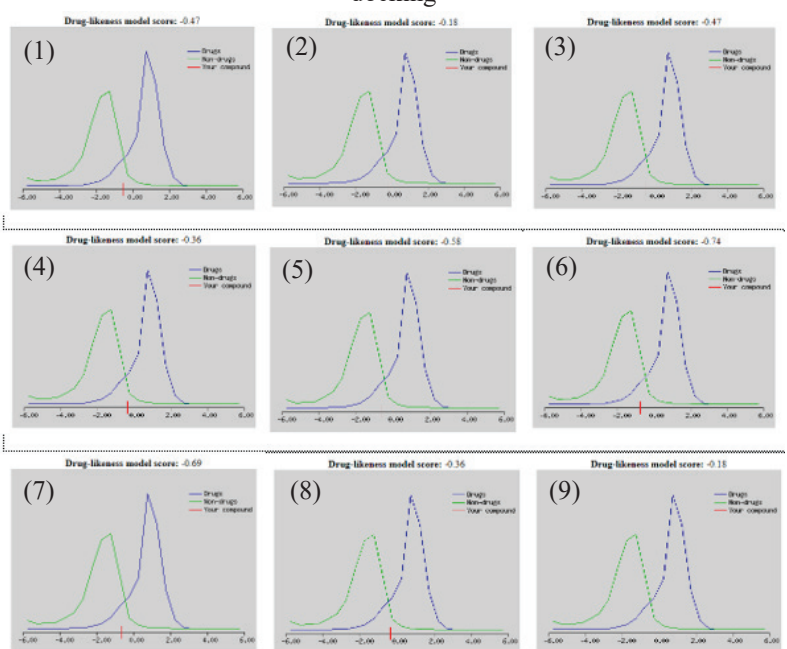


Figure 4: Drug-likeness model score

VI. Acknowledgement

The authors wish to thank Universiti Teknologi Malaysia and the Ministry of Higher Education (MOHE) Malaysia for funding this research under the Fundamental Research Grant Scheme (FRGS/1/2022/stg04/utm/02/4).

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