Synthesis, *in vitro* and *in silico* evaluation of sulfonylurea-chalcone hybrid analogues as α-glucosidase inhibitors

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Abstract
Identification of novel α-glucosidase inhibitors is in urgent demand due to the high prevalence and limitations of existing drugs used in the therapeutic management of type 2 diabetes. In line with the need, we synthesised and evaluated a series of sulfonylurea-chalcone hybrid analogues for their bioactive potential as α-glucosidase inhibitors. Based on the in vitro analysis of screening results and structure-activity relationship (SAR), compound c was found to be a promising bioactive hit molecule (IC_{50}: 1.776 μM).

In silico molecular docking studies revealed that the sulfonylurea-chalcone hybrid backbone exhibited stable binding properties at the human α-glucosidase (PDB ID: 3TOP) binding site region.

Keywords: α-Glucosidase, sulfonylurea-chalcone hybrid, type 2 diabetes.

Introduction

Diabetes mellitus (DM) is a metabolic disorder that affects about 9.3% (463 million people) of the population over the world in 2019, according to the International Diabetes Federation (IDF). It is estimated that the cases of diabetes will increase drastically to 10.9% (700 million people) of the population by 2045[5]. The most common DM diagnosed in patients is type-2 Diabetes Mellitus (T2DM), which is characterised by either insulin resistance or reduced insulin production by beta-cells or both and results in abnormal hyperglycemia. There are a few types of antidiabetic drugs available on the market currently such as metformin, sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, to manage patients' blood glucose levels.

However, there are some side effects such as hypoglycemia and weight gain, with the current medication. Hence, exploration of new antidiabetic drugs is still under extensive study[13]. α-Glucosidase (α-D-glucoside glucohydrolase E.C. 3.2.1.20) is an enzyme located in the brush border of the small intestine. It catalyses the hydrolysis of α-glucosidic linkages in oligosaccharides, producing D-glucose residues that are absorbed in the intestine. It is responsible for the elevation of blood glucose levels. α-glucosidase inhibitors bind competitively to these enzymes to slow down carbohydrate digestion, thus delaying glucose absorption. It is used to decrease postprandial blood glucose in T2DM patients. Current α-glucosidase inhibitors that are available on the market are acarbose, miglitol and voglibose[10].

Sulfonylureas compound is a class of antidiabetic medication[2]. Besides antidiabetic properties, they possess a wide range of biological activities such as antihyperglycemia, cytotoxic and antimicrobial. Some of the sulfonylureas that are used in clinical settings are glipizide, glibenclamide and gliclazide[6]. They decreased blood glucose levels by stimulating insulin secretion by pancreatic beta cells. However, it may cause some unwanted effects such as hypoglycemia and weight-increasing effects[11]. Studies have shown that combination therapy of α-glucosidase and sulfonylureas can minimise these side effects as well as can achieve good glycaemic control for a prolonged period[12]. Chalcone belongs to a flavonoid family possessing a 1, 3-diphenyl-2-propen-1-one structure[15].

It displayed several biological activities such as antitumor, anti-inflammatory, anticancer, antioxidant, antiproliferative, antimicrobial, antiviral, antibacterial, antimycobacterial etc. Molecular hybridisation is a concept in modern medicinal chemistry that combines different active pharmacophores, resulting in a new scaffold identification with improved efficacy when compared to the parent drug[8]. Molecular hybridisation is a technique of drug discovery implemented in this study, hybridising sulfonylurea and chalcone pharmacophore, forming a novel chemotype with α-glucosidase inhibitory properties. This study consists of synthesis, *in vitro* and *in silico* biological evaluation of sulfonylurea-chalcone hybrid analogues.

Material and Methods

Instruments: The reaction progress and compound's purity were checked on pre-coated 60 F254 silica gel TLC plates (USA, Merck, 0.25 mm) thickness by means of a gradient solvent system with n-hexane and ethyl acetate. The electron ionisation mass spectra (ESI-MS) were recorded using mass spectrometry. Melting points (Apparatus United Kingdom, Stuart Scientific, Model: SMP1) were determined in open capillary tubes and were uncorrected. Schrodinger Drug Discovery software and computer hardware facilities were used.

Chemicals: All the regents and chemicals were purchased from Sigma-Aldrich, USA; these include p-toluenesulfonyl isocyanate, 3-aminoacetophenone, benzaldehyde, 2-chlorobenzyaldehyde, 3-chlorobenzyaldehyde, 4-

chlorobenzyaldehyde, 2-fluorobenzyaldehyde, 3-fluorobenzyaldehyde, 4-fluorobenzyaldehyde, 2-bromobenzyaldehyde, 3-bromobenzyaldehyde, 4-bromobenzyaldehyde, 2,4-dimethoxybenzladehyde, 2,5-dimethoxybenaldehyde, 2,4,5-trimethoxybenzaldehyde, anhydrous sodium sulphate, ethanol, acetone, methanol, chloroform and dichloromethane respectively[4].

General procedure for synthesizing intermediates (a): To a solution of o-amino acetophenone (0.01 M) dissolved in 20 mL of dry dichloromethane (CH_2Cl_2), tosyl isocyanate (0.015 M) was added, quickly by syringe and the resulting mixture was stirred at room temperature for 10 min. The reaction mixture was then heated under reflux for 30 min. and then cooled or evaporated to isolate the product. The crude 1-(3-acetylphenyl)-3-tosylurea (a) was washed on the vacuum filter with cold dichloromethane and then recrystallised from ethanol. Reaction scheme is shown in figure 1.

General procedure for the synthesis of sulfonylurea-hybrid analogues (b-n): To a solution of 1-(3-acetylphenyl)-3-tosylurea (a) (0.005 M) and suitably substituted aldehydes (0.005 M) in ethanol (10 ml), an aqueous solution of potassium hydroxide (100%) was added dropwise with continuous stirring at room temperature over a period of 10 minutes. The reaction mixture was then kept at room temperature for about 48 h with occasional shaking. After 48 h, it was poured into ice-cold water and then neutralised to pH 2 using 5 N hydrochloric acid. The yellow precipitate obtained was filtered, washed, dried and recrystallised from dry ethanol. The sulfonylurea-chalcone hybrid analogues (b-n) were obtained. Reaction scheme is shown in figure 1.

General procedure of *in vitro* α-glucosidase inhibitory assay: α-Glucosidase inhibitory properties of compounds (a-n) were measured using *in vitro* enzymatic kinetics bioassay (Figure 1). The solutions (Sigma-Aldrich, USA) that were used on this biological evaluation, were enzyme solutions from *Saccharomyces cerevisiae* type 1, 4-nitrophenyl α-D-glucopyranoside as substrate, voglibose as standard, phosphate buffer (pH 7.3) and dimethyl sulfoxide (DMSO). This inhibition assay was conducted by a total 130 µL volume of different working solutions including reaction control (Enzyme: 120 µL, Phosphate buffer: 5 µL, Phosphate buffer + Substrate: 5 µL), reaction control-blank (Enzyme: 120 µL, Phosphate buffer: 10 µL), reaction test (Enzyme: 120 µL, DMSO + Test compound: 5 µL, Phosphate buffer + Substrate: 5 µL), reaction solvent blank (Enzyme: 120 µL, DMSO: 5 µL, Phosphate buffer + Substrate: 5 µL), reaction standard (Enzyme: 120 µL, Phosphate buffer + Substrate: 5 µL, DMSO + Voglibose: 5 µL (100 µM to 0.5 µM).

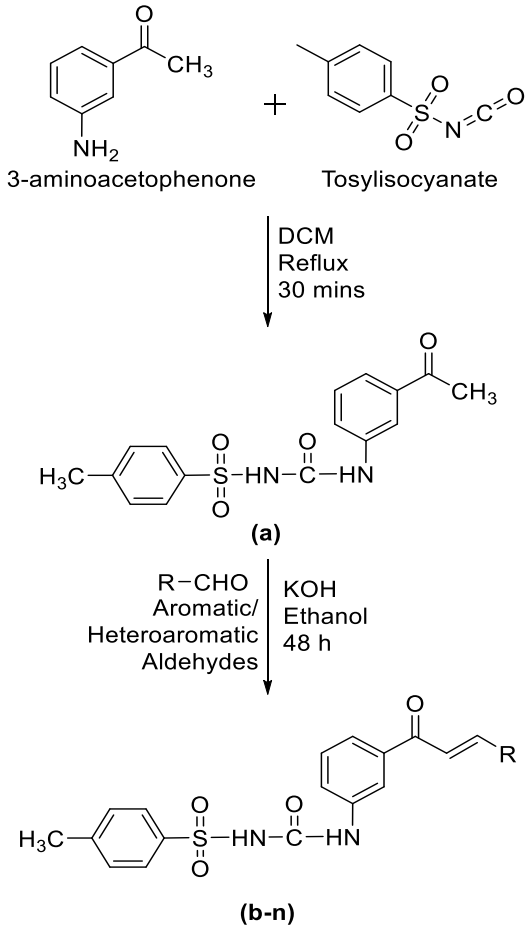
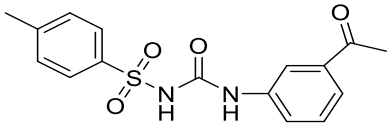
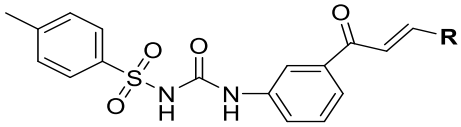


Figure 1: Synthetic reaction scheme of sulfonylurea-chalcone hybrid analogues (a-n)

Table 1
Melting point and relative molecular mass of compounds (a-n)



Intermediate (a)



Compounds (b-n)

Code	R	Melting Point (ºC)	Relative Molecular Mass (RMM)	Electrospray Ionisation mode	Observed mass to charge ratio (m/z)	Molecular adduction
a	Intermediate	173-177	332	Positive	333.10	$[M+H]^+$
					355.13	$[M+Na]^+$
				Negative	330.98	$[M-H]^-$
b	C_6H_5	139-142	420	Positive	421.15	$[M+H]^+$
					443.27	$[M+Na]^+$
				Negative	419.11	$[M-H]^-$
c	2-ClC_6H_4	166-168	454	Positive	476.98	$[M+Na]^+$
					478.96	$[M+Na]^+$
				Negative	453.13	$[M-H]^-$
					455.12	$[M-H]^-$
d	3-ClC_6H_4	163-165	454	Positive	477.02	$[M+Na]^+$
					478.97	$[M+Na]^+$
				Negative	453.14	$[M-H]^-$
					455.11	$[M-H]^-$
e	4-ClC_6H_4	165-167	454	Positive	477.01	$[M+Na]^+$
					479.01	$[M+Na]^+$
				Negative	453.17	$[M-H]^-$
					471.16	$[M-CH_4]^-$
f	2-FC_6H_4	90-92	438	Positive	461.11	$[M+Na]^+$
				Negative	437.02	$[M-H]^-$
g	3-FC_6H_4	133-135	438	Positive	461.24	$[M+Na]^+$
				Negative	437.22	$[M-H]^-$
h	4-FC_6H_4	101-104	438	Positive	439.07	$[M+H]^+$
					461.10	$[M+Na]^+$
				Negative	437.11	$[M-H]^-$
i	2-BrC_6H_4	142-145	499	Positive	523.37	$[M+Na]^+$
					520.92	$[M+Na]^+$
				Negative	499.11	$[M-H]^-$
					497.06	$[M-H]^-$
j	3-BrC_6H_4	216-218	499	Positive	500.15	$[M+H]^+$
				Negative	499.10	$[M-H]^-$
					497.01	$[M-H]^-$
k	4-BrC_6H_4	132-135	499	Positive	522.84	$[M+Na]^+$
					520.84	$[M+Na]^+$
				Negative	499.07	$[M-H]^-$
					497.02	$[M-H]^-$
l	2,4-di(OCH_3)C_6H_3	74-77	499	Positive	485.29	$[M+H]^+$
					503.22	$[M+Na]^+$
				Negative	479.29	$[M-H]^-$
m	2,5-di(OCH_3)C_6H_3	90-92	499	Positive	480.91	$[M+H]^+$
					503.19	$[M+Na]^+$
				Negative	479.30	$[M-H]^-$
n	2,4,5-tri(OCH_3)C_6H_2	86-89	510	Positive	511.60	$[M+H]^+$
					533.41	$[M+Na]^+$
				Negative	509.37	$[M-H]^-$

All the solutions were incubated for 20 minutes, then the absorbance was measured at 405 nm. The IC_{50} values were determined using concentrations (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.01 µM) of compounds (a-n) in the inhibition of 50% of the α-glucosidase enzyme activity under the assay conditions. Microsoft Excel was used for data analysis. Data is expressed as mean ± standard error of mean (SEM). Enzyme inhibition percentage (%) has been calculated based on the formula (1- "Absorbance of test compound - Absorbance of solvent blank"/"Absorbance of control - Absorbance of control blank") x 100. Statistical analysis was carried out using Microsoft Excel[13].

General procedure of *in silico* molecular docking studies: *In silico* molecular docking[1] was carried out by using Schrödinger Maestro 11.3 software. Initially, a selected molecular target of the crystal structure of the C-terminal subunit of human maltase-glucoamylase in complex with acarbose (PDB ID: 3TOP) was retrieved from the RCSB Protein Data Bank[7]. Water molecules were removed and any missing atom and bond valence were added to the protein structure before energy minimisation with the Protein Preparation Wizard[9]. After that, the binding site of the energy minimised protein-ligand complex was identified as a region at center_x: -30.67, center_y: 35.56 and center_z: 26.61 by the receptor grid generation (Glide) application. The 2-D sketchers were employed to draw the ligand structures and the LigPrep application was utilised for ligand preparation[3]. The prepared ligands were then docked to the binding site of protein through ligand docking (Glide) under high throughput virtual screening (HTVS). Docking scores for each ligand binding were obtained after the molecular docking[14].

Results and Discussion

A series of characterisations of compounds synthesised was done after purification processes. Each compound synthesised was subjected to melting point determination and mass analysis. The mass spectral data of the synthesised compounds were determined using Electrospray Ionisation Mass Spectrometry (ESI-MS) in positive and negative ion modes using methanol as solvent (Table 1).

For compound a (intermediate), the positive ion ESI mass spectrum revealed a pseudo-molecular ion at m/z 333.10 as the base peak, indicative of a [M+H]+ molecular ion. A sodium adduction peak was observed at m/z 355.13, representing [M+Na]+ molecular ion. Likewise, the negative ion ESI mass spectrum revealed a pseudo-molecular ion at m/z 330.98 as the base peak, indicative of a $[M–H]^-$ molecular ion. Both the molecular ions was corresponded to the relative molecular mass (RMM) of 332.37, formula $C_{16}H_{16}N_2O_4S$. RMM of the benzaldehyde derivative was 420.48 calculated from the molecular formula of $C_{23}H_{20}N_2O_4S$. The positive ion ESI mass spectrum revealed a pseudo-molecular ion at m/z 421.15 as the base peak, indicative of [M+H]+ molecular ion. A sodium adduction peak was observed at m/z 443.27, presenting [M+Na]+ molecular ion. For the negative ion ESI mass spectrum, a base peak was observed through deprotonation of the compound-forming $[M–H]^-$ molecular ion and this was shown at m/z 419.11. Chlorine substituted derivatives were calculated with an RMM of 454.93 from the molecular formula of $C_{23}H_{19}ClN_2O_4S$. Two sodium adduction peaks were observed, presenting [M+Na]+ molecular ion at m/z 476 and 478. Likewise, the negative ion ESI mass spectrum revealed pseudo-molecular ion at m/z 453 and 455 as base peaks, indicative of $[M–H]^-$ molecular ion. Two pseudo molecular ion peaks were consistently formed due to isotopic properties of ^{35}Cl and ^{37}Cl.

A methane adduction was observed for chlorine substituted at para position at m/z 471.16, which indicates the formation of the $[M\text{-}CH_4]$- molecular ion. For fluorine substituted derivatives, RMM calculated was 438.47 with corresponding chemical formula is $C_{23}H_{19}FN_2O_4S$. The positive ion ESI mass spectrum revealed a pseudo-molecular ion at m/z 439 as the base peak, indicative of a [M+H]+ molecular ion. Similarly, a pseudo-molecular ion base peak was observed in the negative ion ESI mass spectrum at m/z 437, presenting $[M–H]^-$ molecular ion. Bromine substituted derivatives with the chemical formula $C_{23}H_{19}BrN_2O_4S$ had a calculated RMM of 499.38. Two sodium adduction peaks were observed at the positive ion ESI mass spectrum for ortho and para substitution of bromine derivatives at m/z 522 and 520, revealing [M+Na]+ molecular ions.

A base peak indicating [M+H]+ molecular ion was observed at m/z 500.15. For the negative ion ESI mass spectrum, pseudo-molecular ions at m/z 499 and 497 as base peaks were observed, indicative of a $[M–H]^-$ molecular ion formation from deprotonation. The isotopic property of the bromine compound can be observed through the spectrum showing M and M+2 due to the presence of $_{79}Br$ and $_{81}Br$ with an approximately 1:1 ratio. For 2,4 and 2,5 dimethoxy substituted derivatives, RMM calculated from molecular formula was 480 with chemical formula of $C_{25}H_{24}N_2O_6S$. The positive ion ESI mass spectrum revealed a pseudo-molecular ion at m/z 495 as a base peak, indicative of [M+H]+ molecular ion.

A sodium adduction peak was observed at m/z 503, presenting [M+Na]+ molecular ion. For the negative ion ESI mass spectrum, a base peak was observed through deprotonation of the compound forming the $[M–H]^-$ molecular ion and this was shown at m/z 479. The chemical formula of the 2,4,5 trimethoxy substituted derivative was $C_{26}H_{26}N_2O_7S$ with an RMM of 510.56. Positive ion ESI mass spectrum shows base peak of [M+H]+ molecular ion at m/z 511.60 and sodium adduction, [M+Na]+ molecular ion at m/z 533.41. Besides, negative ion mode spectra have revealed the formation of $[M–H]^-$ molecular ions at a base peak of m/z 509.37. All compounds were carried forward to *in vitro* studies.

In vitro screening, the IC_{50} value was determined by the

amount of synthesised compound needed to inhibit 50% of the specific biological or biochemical function. IC_{50} values are commonly used to evaluate the inhibitory potency of compounds to a specific enzyme. In this research, the inhibitory activity of compounds towards α-glucosidase is determined using the IC_{50} value generated from a graph of log concentration versus normalised absorbance in percentage. Results of IC_{50} values obtained from synthesised compounds are shown in table 2.

Compound 2-chlorobenzaldehyde derivative was identified as the hit molecule with an IC_{50} value of 1.776 µM followed by 2-fluorobezaldehyde derivative with an IC_{50} value of 1.784 µM. The antihyperglycemic properties of compounds were contributed by the alpha beta unsaturated ketone and sulfonylurea linker moiety. However, the antihyperglycemic effect of the compound was shown to be enhanced by the substitution of chlorine, fluorine, bromine and methoxy groups to the phenyl ring. Hence, structure activity relationship (SAR) studies have been successfully established.

Table 2
***In vitro* α-glucosidase inhibitory properties of compounds (a-n)**

Code	R	IC_{50} (µM)
a	Intermediate	2.481
b	C_6H_5	2.543
c	2-ClC_6H_4	1.776
d	3-ClC_6H_4	2.679
e	4-ClC_6H_4	2.094
f	2-FC_6H_4	1.784
g	3-FC_6H_4	2.204
h	4-FC_6H_4	>100
i	2-BrC_6H_4	2.174
j	3-BrC_6H_4	2.560
k	4-BrC_6H_4	>100
l	2,4-di(OCH_3)C_6H_3	2.976
m	2,5-di(OCH_3)C_6H_3	>100
n	2,4,5-tri(OCH_3)C_6H_2	>100
Standard (Voglibose)		0.36

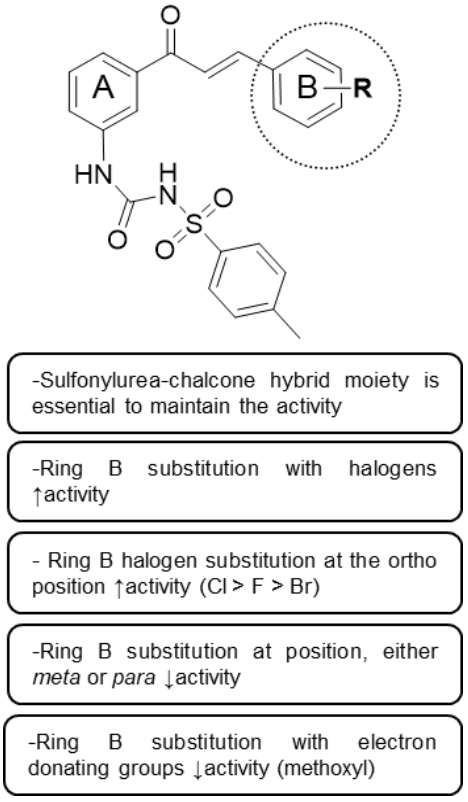


Figure 2: Structure-activity relationship (SAR) analysis of sulfonylurea-chalcone hybrid analogues with respect to their *in vitro* α-glucosidase inhibitory properties

Among the group of substituents, chlorine substitution has shown to increase the inhibitory potency of the synthesised derivatives towards alpha glucosidase following the order of ortho > para > meta. This order of inhibition was aligned with research findings where the ortho position was shown to enhance best the inhibitory property towards alpha glucosidase. For bromine and fluorine substituted derivatives, inhibitory potency was shown to follow the order of ortho> meta> para. This order can be seen clearly with the reported IC_{50} values of 2-Br derivative (2.174 µM), 3-Br derivative (2.560 µM) and 4-Br derivative (>100 µM).

For fluorine substituted derivatives, the IC_{50} value of 2-F derivative is 1.784 µM, followed by 3-F derivative (2.204 µM) and 4-F derivative (>100 µM). The 2,4-diMeO derivative was found to have the least potent inhibitory effect on alpha-glucosidase, with a reported IC_{50} value of 26.22 µM. Methoxy substitution was shown to be not favourable in alpha glucosidase inhibition. The overall inhibitory potency of synthesised compounds follows the order of 2-Cl > 2-F > 4-Cl > 2-Br > 3-F > intermediate > benzaldehyde > 3-Br > 3-Cl > 2,4-diMeO. The SARs are derived based on the observed bioactivity as depicted in figure 2.

In silico molecular docking study was carried out by using a computational method to investigate the binding orientation and the binding affinity of synthesised compounds (a-n) in a simulated protein environment.

Table 3
***In silico* ligand binding interactions profile of compounds (a-n) at the 3TOP target binding site region.**

Code	Types of binding interactions	Frequency	Binding site amino acid residues	Docking score (kcal/mol)
a	(i)Pi-Pi stacking (ii)H-bond (iii)Salt bridge	(i)1 (ii)1 (iii)1	(i)Trp1355 (ii)Lys1460 (iii)Lys1460	-3.607
b	(i)Pi-Pi stacking (ii)H-bond	(i)2 (ii)1	(i)Phe1560, (ii)Trp1355 (iii)Gln1561	-3.556
c	(i)Pi-Pi stacking (ii)H-bond (iii)Salt bridge	(i)1 (ii)1 (iii)1	(i)Phe1560 (ii)Arg1510 (iii)Lys1460	-3.785
d	(i)Salt bridge	(i)1	(i)Lys1460	-3.060
e	(i)Pi-Pi stacking (ii)H-bond (iii)Pi-cation	(i)2 (ii)1 (iii)1	(i)Trp1355, (ii)Phe1559 (iii)Arg1510 (iv)Lys1460	-4.105
f	(i)Salt bridge	(i)1	(i)Lys1460	-3.957
g	(i)H-bond (ii)Salt bridge	(i)1 (ii)1	(i)Lys1460 (ii)Lys1460	-4.009
h	(i)Pi-Pi stacking (ii)H-bond (iii)Salt bridge	(i)1 (ii)1 (iii)1	(i)Phe1559 (ii)Lys1460 (iii)Lys1460	-3.826
i	(i)H-bond	(i)1	(i)Gly1588	-3.775
j	-	-	-	-4.174
k	(i)Pi-Pi stacking (ii)H-bond (iii)Salt bridge	(i)2 (ii)1 (iii)1	(i)Tyr1251, (ii)Phe1559 (iii)Lys1460 (iv)Lys1460	-3.844
l	(i)Pi-Pi stacking (ii)H-bond (iii)Salt bridge	(i)3 (ii)1 (iii)1	(i)Trp1369, (ii)Trp1355 (iii)Lys1460 (iv)Lys1460	-2.824
m	(i)Pi-Pi stacking (ii)H-bond (iii)Salt bridge	(i)1 (ii)2 (iii)1	(i)Phe1560 (ii)Lys1460 (iii)Lys1460	-3.239
n	(i)H-bond (ii)Salt bridge (iii)Pi-cation	(i)1 (ii)1 (iii)1	(i)Gln1158 (ii)Lys1460 (iii)Lys1460	-3.041

The 3D crystallographic structure (PDB ID: 3TOP) was utilised to mimic the binding environment of the α-glucosidase enzyme in the human body. The results are displayed in table 3 and the α-glucosidase enzyme inhibitory activity was determined by their docking scores.

The lower is the docking score, the better fit of the compound is in the binding site and hence, predicts a greater inhibitory effect of the compound. The interactions of amino acid residue with compounds (a-n) mainly involved Pi-Pi stacking, H-bond, salt bridge and Pi-cation. Compound j had the lowest docking score -4.174 with the absence of bond interaction. This was followed by compound e (-4.105) and g (-4.009). Compound e interacted with Trp1355 and Phe1559 via Pi-Pi stacking, H-bonding with Arg1510 and pi-cation with Lys1460, while compound g formed an H-bond and salt bridge with Lys1460. Compound l had the highest docking score -2.824 which involved a total of three Pi-Pi stacking interactions with Trp1369 and Trp1355 residues and one H-bond and salt bridge with Lys1460.

Compound f possessed a docking score of -3.957 and formed a salt bridge with Lys1460. Compounds h and k had similar docking scores which were -3.826 and -3.844 respectively. Both of the compounds formed an H-bond and a salt bridge with Lys1460 residues, besides a Pi-Pi stacking with Phe1559 in compound h and two Pi-Pi stacking with Tyr1251 and Phe1559 in compound k. The other two compounds that had a close docking score, were compounds c (-3.785) and i (-3.775). Compound C interacted to have Pi-Pi stacking with Phe1560, an H-bond with Arg1510 and a salt bridge with Lys1460, whereas compound I only had an H-bond with Gly1588. Compound a (-3.607) was observed with Pi-Pi stacking in Trp1355, H-bond and salt bridge in Lys1460.

Compound b (-3.556) had two Pi-Pi stacking in Phe1560 and Trp1355 residues and a H-bond in Gln1561 residue. Compound m (-3.239) docked in the binding site to form a Pi-Pi stacking with Phe1560, two H-bonds and a salt bridge with Lys1460. There was a salt bridge linked with Lys1460 residue in compound d which resulted in a docking score of -3.060. Compound n (-3.041) fitted into the binding site with an H-bond on Gln1158 residue, a salt bridge and a pi-cation on Lys1460 residue. Hence, Pi-Pi stacking, H-bond and salt bridge interactions played a vital role in the ligand-protein interaction of the study and Lys1460 was the most common observed residue among the interactions.

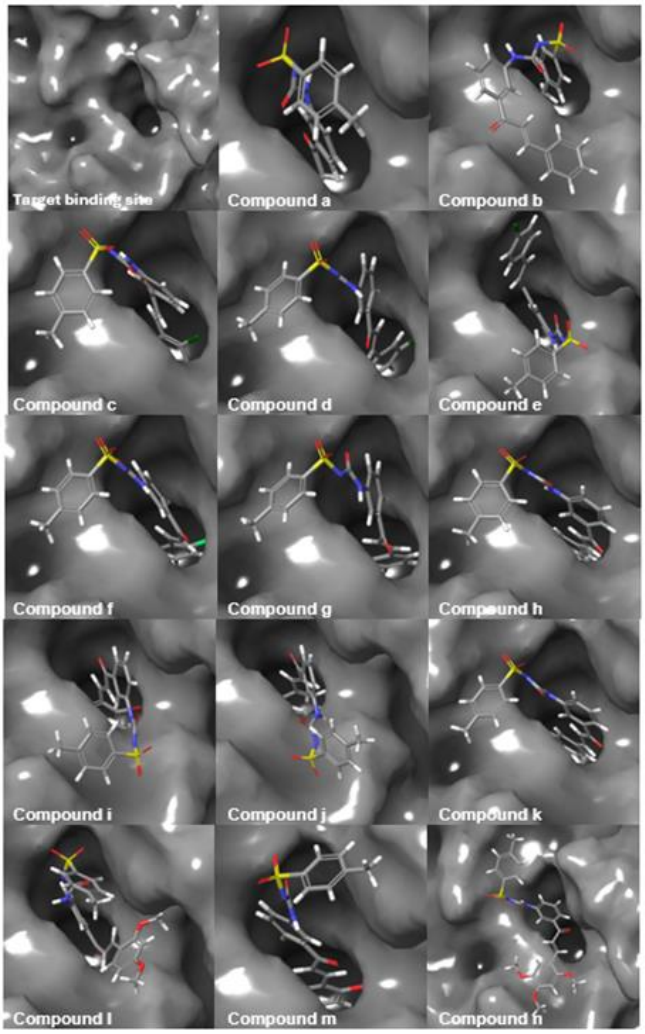


Figure 3: *In silico* ligand binding orientation of compounds (a-n) at the 3TOP target binding site region

It was important to note that although compounds a, c and h had the same types and number of interactions, their docking scores varied from -3.607 to -3.785 and -3.826 respectively, as the residues involved and their different binding orientations in the active site of the protein were different. The 3D binding orientation of compounds a-n at the target binding site region is shown in figure 3. Generally, the nature of experimental and simulated systems contributes to a lack of established good correlation between *in vitro* and *in silico* studies.

Conclusion

In summary, our study highlights an exciting new approach in drug discovery through molecular hybridization, which combines the beneficial properties of sulfonylureas and chalcones to create a new class of α-glucosidase inhibitors. One of the major findings is the remarkable *in vitro* inhibitory activity of compound c which shows great promise as a potential to carry forward to animal studies. Our *in silico* molecular docking studies also provided valuable insights into how these hybrid compounds interact with the human α-glucosidase enzyme, indicating that they can form stable and favourable interaction at the target site region.

Additionally, our research points to the need for further studies to refine these hybrid compounds, in relation to their pharmacokinetic and pharmacodynamic properties. It is crucial to conduct acute toxicity evaluations to ensure the safety and therapeutic potential of these compounds in real biological settings. Overall, this research adds to our ongoing efforts in drug discovery for type 2 diabetes, showcasing the promise of hybrid compounds in developing more effective α-glucosidase inhibitors.

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