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Biochemical Interaction Analysis of Natural SGLT2 Inhibitors with Alzheimer Targets: A Computational Approach

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Abstract

Alzheimer's Disease (AD) is a progressive neurological disorder, and its association with type 2 diabetes mellitus (T2DM) has increased its intricacy to many folds. Thus, targeting both associated disorders with a single therapeutic agent appears to be promising. Anti-AD potency of a new class of anti-diabetic agents, i.e., Sodium-Glucose Co-transporters 2 (SGLT2) inhibitors have been investigated in the present study. The docking approach was applied to elucidate the interaction pattern of natural SGLT2 inhibitors (Acerogenin A, Alstiphyllanine D, ε-viniferin, Phlorizin, and Sophoraflavanone G) with AD targets. Here, Acerogenin A, ε-viniferin and Sophoraflavanone G showed better binding with almost all AD targets as compared to positive controls, except β-secretase where positive control verubecestat showed better results than these inhibitors. Also, ε-viniferin and Sophoraflavanone G have shown potency against direct amyloidβ aggregation. We state that SGLT2 inhibitors based scaffolds could be explored to develop dual therapeutic agents against T2DM and AD.

Keywords: Alzheimer's Disease, Dual Therapy, Molecular Docking, Sodium-Glucose Co-transporters 2, Type 2 diabetes mellitus.

Introduction

Alzheimer's Disease (AD) is an irreversible and progressive disorder of the central nervous system that is considered the major reason for dementia in the elderly population worldwide (Alharthy, et al., 2019; Abdulaziz, et al., 2019; Osman, et al., 2019; Ahmed, et al., 2018). It has been stated in the World Alzheimer's Report 2018 (https://www.alz.co.uk/research/WorldAlzheimerReport2018.pdf.) that around 50 million individuals are currently suffering from AD and another type of dementia; however, by 2050 the number is expected to reach approximately 152 million. AD's strong association with other diseases such as type 2 diabetes mellitus (T2DM) has added to its intricacy (Rizvi et al., 2015; Caberlotto et al., 2019). T2DM is one of the focal predisposing factors for AD and in fact, reports suggest that it can increase the risk of AD by 1.6 fold (Sonnen et al., 2009; McIntosh et al., 2019). The situation for diabetes is graver than AD, around 425 million diabetic patients are reported in the statistics of the International Diabetes Federation 2017 (https://diabetesatlas.org/IDF_Diabetes_Atlas_8e_interactive_EN/.) and this might reach 629 million by 2045. The death rate for diabetes has reached one in seven seconds with 50% diabetic deaths in the age group of fewer than 60 years. Failure to treat both these debilitating diseases is a matter of great concern to the scientific community globally. Thus, researchers are trying to cope-up with the situation with alternate upcoming strategies. One such strategy is to treat T2DM with Sodium-glucose co-transporter 2 (SGLT2) inhibitors that have been approved by the FDA in the year 2013 (https://www.fda.gov/media/87579/download). SGLT2 is present in the proximal convoluted tubules of kidneys that are accountable for ~90% reabsorption of filtered glucose. Subsequently, SGLT2 inhibition will reduce the reabsorption of glucose and considered as a target for T2DM therapy (Scheen et al., 2015; Fioretto et al., 2015; Hsia et al., 2017).

In the present study, natural SGLT2 inhibitors were explored for their efficacy against AD. Even though the main targeted organs of T2DM and AD i.e., pancreas and brain are physiologically different and anatomically distant, they share some common pathophysiology (Rizvi et al., 2015; Caberlotto et al., 2019). It has been evident from various reports that pancreatic islet amyloid polypeptide could enter the brain and plausibly initiate misfolding of amyloidβ, amyloid plaque formation and neuronal degradation during AD (Oskarsson et al., 2015; N Fawver et al., 2014; de la Monte et al., 2008). As T2DM could initiate the onset of AD, a treatment regime focused on dual therapeutic agents might plausibly provide a robust alternative to current unavailing medications. Five natural SGLT2 inhibitors, namely, Acerogenin A, Alstiphyllanine D, ε-viniferin, Phlorizin, and Sophoraflavanone G (Sato et al., 2007; Oranje et al., 2019; Choi et al., 2016; Morita et al., 2010; Arai et al., 2010) were chosen against AD targets. AD is a multifaceted disease involving various suggested hypotheses. Among all AD hypotheses, the most accepted are cholinergic, amyloidogenic, and tau phosphorylation pathways. In this study, initially, the physiochemical properties and toxicity potential of natural SGLT2 inhibitors were checked followed by analyzing the interaction pattern of these natural inhibitors against SGLT2. Afterward, the prominent three pathways via targeting 'Acetylcholinesterase', 'β–secretase', 'Amyloidβ aggregation', 'Fyn Kinase', 'Glycogen synthase kinase 2 beta' and 'Tau Tubulin Kinase 1' were covered. For all the assessments, control compounds were used for each target, such as empagliflozin for SGLT2, galantamine for acetylcholinesterase, verubecestat for β–secretase, curcumin for amyloid aggregation, leuco-methylthioninium for fyn kinase, glycogen synthase kinase 2 beta, and tau tubulin kinase

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1, respectively. Interestingly, the results were quite promising and fascinating. Moreover, these predicted findings would pave the way for future dual therapeutic agents in the form of SGLT2 inhibitor scaffold for T2DM associated AD.

Materials and Methods

Sodium-glucose co-transporter 2 (SGLT2) inhibitors, control compounds, and target proteins structure retrieval

The 3-dimensional structures of natural SGLT2 inhibitors, namely, Acerogenin A [CID: 12000158], Alstiphyllanine D [CID: 45269680], ε-viniferin [CID: 5281728], Phlorizin [CID: 6072] and Sophoraflavanone G [CID: 73198] and control compounds Empagliflozin [CID: 11949646], Galantamine [CID: 9651], Verubecestat [CID: 51352361], Curcumin [CID: 969516] and Leuco-Methylthioninium [CID: 122173994] were retrieved from PubChem database. However, target protein structures of Acetylcholinesterase (AChE) [ID: 3LII], β-secretase (BACE-1) [ID: 1W51], β-turn-β-fold of $A\beta_{1–42}$ peptide ($A\beta_{17-42}$) [ID: 2BEG], Fyn Kinase (Fyn) [ID: 2DQ7], Glycogen synthase kinase 2 beta (GSK3β [ID: 1J1C] and Tau Tubulin Kinase 1(TTBK1) [ID: 4BTJ] were obtained from Protein Data Bank. However, the swiss model workspace was used to prepare the 3-D structure of the SGLT2 protein after retrieving the amino acid sequence from Uniprot [P31639].

Calculation of physicochemical properties and prediction of toxicity potential

Orisis Datawarrior property explorer tool was used (http://www.openmolecules.org/datawarrior/download.html) to check the physicochemical properties and toxicity potential of all the compounds including control. The number of hydrogen bond donors and acceptors, molecular weight, cLogP value, molecular weight, topological polar surface area, number of rotatable bonds, and the Lipinski's rule violation (Lipinski et al., 2001) were assessed using the orisis software (Table 1). Also, Absorption % was calculated according to the method of Zhao et al. (Zhao et al., 2002) by applying the formula % of Absorption = 109 – (0.345 × TPSA). In the orisis data warrior tool, toxicity potential predictions are dependent on the comparative analysis of a pre-calculated set of already investigated structural molecules with the structure of tested compounds. The software predicted various aspects of toxicity such as mutagenicity, tumorigenicity, reproductive effects, and irritability of all the tested compounds (Table 2).

Molecular Docking

The protocol of MD Rizvi et al. (2013) was used to perform docking of all the tested compounds with the target protein. The MMFF94 force field was used for energy minimization of each compound followed by the addition of gasteiger partial charges. After the inclusion of non-polar hydrogen atoms, rotatable bonds were defined. AutoDock was used to add solvation parameters, hydrogen atoms, and Kollman united atom type charges. The dimensions of the grid were kept as 60 x 60 x 60 Å with points separated by 0.375 Å by using an Auto grid. To target specifically, the values of x, y and z coordinates were kept as 90.81, 83.98 and -8.04 for AChE; 73.79, 54.27 and 11.51 for BACE-1, -19.093, 25.139 and -11.588 for Fyn, 21.613, 17.277, -8.568 for GSK3β and 32.866, 38.648, -2.784 for TTBK1, respectively. For SGLT2, the grid center was kept at various well-recognized amino acid residues to dock the compounds. Default parameters and dielectric functions of AutoDock were used for van der Waals and the calculation of the electrostatic term. Docking simulation was performed using 'Solis and Wets local search method' and 'Lamarckian genetic algorithm'. Other parameters of docking simulations such as orientation, initial position, and torsions were set arbitrarily. For each docking experiment, 100 different runs were applied for that was set to end after 2,500,000 energy evaluation. The population size was set as 150. Discovery Studio 2.5 (Accelrys) was used to create the final figures of AutoDock 4.2

The anti-aggregation potential of compounds against β-turn-β-fold of $A\beta_{1–42}$ peptide was performed by using Hex 5.1 tool. Compound bounded $A\beta_{17–42}$ peptide and unbounded $A\beta_{17–42}$ peptide were docked with each other. The lowest E-total evaluation in Hex was based on 'shape only' correlation type. The first Fourier transform (FFT) model was selected as 3D Fast Lite, the grid dimension was adjusted to 0.75, and the rest of the parameters were kept as default.

Results and Discussion

Alzheimer's Disease (AD) and Type 2 Diabetes Mellitus (T2DM) both are intricate diseases with a strong correlation between them. There are several epidemiological, clinical, and pathophysiological pieces of evidence that validate the linkage between AD and T2DM (Rizvi et al., 2015; Caberlotto et al., 2019; Sonnen et al., 2009; McIntosh et al., 2019). Autopsy samples of T2DM patient's brains have shown amyloid plaque accumulation, and it has been observed that insulin resistance and hyperinsulinemia lead to the building up of these amyloid plaques (Peila et al., 2002; Matsuzaki et al., 2010). Also, it has been reported that uncontrolled T2DM diabetes would increase the chances of AD by 1.6-fold (Sonnen et al., 2009; McIntosh et al., 2019). To date, no breakthrough AD treatment strategy has yet been found. Available AD drugs such as acetylcholinesterase inhibitors and N-methyl D-aspartate receptor antagonist offer merely symptomatic relief to AD patients. Although at present 132 anti-AD agents are under scanner via clinical trials, our past failure rate is too high (Cummings et al., 2019). To get the actual benefit, AD treatment should be started at prodromal or even pre-clinical stages. As T2DM is a prominent predisposing factor for AD, if an anti-T2DM drug scaffold would be used to develop anti-AD drugs it might provide a dual therapeutic strategy against both these linked diseases. There were several clinical trials conducted on currently approved T2DM drugs to estimate their anti-AD potential (Risner et al., 2006; Hsu et al., 2011). Unfortunately, no effective dual therapy of this kind has successfully cleared the Phase III clinical trial so far. All this information prompted us to give a try to a new class of anti-diabetic medication Sodium-glucose co-transporter 2 (SGLT2) protein inhibitors.

MD Rizvi et al. (2014) and Shaikh et al. (2016) have observed the anti-AD potential of recently FDA-approved SGLT2 inhibitors i.e., canagliflozin and dapagliflozin, respectively, and revealed some interesting findings. In the present study, natural SGLT2 inhibitors Acerogenin A, Alstiphyllanine D, ε-viniferin, Phlorizin and Sophoraflavanone G (Sato et al., 2007; Oranje et al., 2019; Choi et al., 2016; Morita et al., 2010; Arai et al., 2010) were selected and tested against different AD targets to explore their dual therapeutic potential in detail. Before evaluating their anti-AD potential, the physicochemical properties and toxicity potential were checked of all these natural SGLT2 inhibitors along with control compounds using the Orisis Datawarrior tool. Table 1 shows the results of the physicochemical properties of each tested compound. It has been found that out of all-natural SGLT2 inhibitors, Acerogenin A and ε-viniferin have not violated Lipinski's rule of five with % absorption of 91.85% and 70.91%, respectively. During toxicity potential analysis (Table 2), none of the natural SGLT2 inhibitors showed toxicity except ε-viniferin and Phlorizin that have high and low reproductive toxicity, respectively. However, both ε-viniferin and Phlorizin have no mutagenic, tumorigenic, and irritant effects.

The main focus was to evaluate the anti-AD potential of all the tested natural SGLT2 inhibitors. As natural compounds were taken instead of known approved SGLT2 inhibitor drugs, they were tested first on SGLT2 protein rather than testing them directly on AD targets. Findings revealed that natural SGLT2 inhibitors Acerogenin A, ε-viniferin, and Sophoraflavanone G showed better binding with SGLT2 than control (Empagliflozin) in terms of binding energy (ΔG) and inhibition constant (Ki) (Table 3). ΔG and Ki values for 'Acerogenin A-SGLT2', 'ε-viniferin-SGLT2', 'Sophoraflavanone G-SGLT2', and 'Empagliflozin-SGLT2' interaction were estimated as '-8.67kcal/mol and 444.91nM', '-10.33kcal/mol and 26.64nM', '-8.84kcal/mol and 331.37nM' and '-6.27kcal/mol and 25.40μM', respectively. Structural functional studies have shown that Q457 amino acid residue of SGLT2 protein is involved directly in the reabsorption of sugar (MD Rizvi et al., 2014; Díez-Sampedro et al., 2001; Liu et al., 2009). Interestingly, in this study, all these natural SGLT2 inhibitors have bound to Q457 of SGLT2 protein (Figure 1). After revealing these promising results, they were tested on different AD targets. Most accepted AD hypotheses such as cholinergic, amyloidogenic, and tau phosphorylation were targeted to evaluate the anti-AD potential of the selected natural SGLT2 inhibitors. 'Acetylcholinesterase (AChE)' was selected target for cholinergic pathway and 'β-secretase (BACE1)' and 'beta-amyloid aggregation' were

Table 1: Physicochemical properties of natural Sodium-Glucose co-transporter 2 (SGLT2) inhibitors and control compounds

Compounds	Physiochemical parameters							
	% of Absorption**	Topological Polar Surface Area (Å)2	Molecular Weight	cLogP***	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Number of Rotatable Bonds	Lipinski's Violation
Rule	**-**	**-**	**<500**	**≤5**	**<5**	**<10**	**≤10**	**≤1**
Acerogenin A	91.85	49.69	298.38	4.50	2	3	0	0
Alstiphyllanine D	72.69	105.23	606.67	3.20	0	11	10	2
ε-viniferin	70.91	110.38	454.47	4.82	5	6	4	0
Phlorizin	47.88	177.14	436.41	0.055	7	10	7	1
Sophoraflavanone G	72.00	107.22	424.49	5.83	4	6	6	1
Empagliflozin*	71.52	108.61	450.91	1.64	4	7	6	0
Galantamine*	94.53	41.93	287.35	1.19	1	4	1	0
Verubecestat*	65.48	126.13	409.41	0.48	2	8	3	0
Curcumin*	76.89	93.06	368.38	2.94	2	6	8	0
Leuco-methylthioninium*	93.88	43.81	285.41	3.57	1	3	2	0

*Control drugs/compounds

**Percentage of Absorption (% of Absorption) was calculated by: % of Absorption= 109 – [0.345 × Topological Polar Surface Area]

***Logarithm of compound partition coefficient between *n*-octanol and water

Table 2: Toxicity Potential of natural Sodium-Glucose co-transporter 2 (SGLT2) inhibitors and control compounds

Compounds	Toxicity Risks			
	Mutagenic	Tumorigenic	Reproductive effect	Irritant
Acerogenin A	None	None	None	None
Alstiphyllanine D	None	None	None	None
ε-viniferin	None	None	High	None
Phlorizin	None	None	Low	None

Sophoraflavanone G	None	None	None	None
Empagliflozin*	None	None	None	None
Galantamine*	None	None	None	None
Verubecestat*	None	None	None	None
Curcumin*	None	None	None	None
Leuco-methylthioninium*	High	None	High	High

*Control drugs/compounds

Table 3: Molecular docking results of 'Sodium glucose co-transporter 2 (SGLT2)' interaction with natural SGLT2 inhibitors and control

Compounds	Binding Energy (ΔG)	Inhibition Constant (Ki)
Acerogenin A	-8.67kcal/mol	444.91nM
Alstiphyllanine D	-6.70kcal/mol	12.27μM
ε-viniferin	-10.33kcal/mol	26.64nM
Phlorizin	-3.79kcal/mol	1.67mM
Sophoraflavanone G	-8.84kcal/mol	331.37nM
Empagliflozin	-6.27kcal/mol	25.40μM

selected targets for amyloidogenic pathway, while, 'Fyn Kinase (Fyn)', 'Glycogen synthase kinase 2 beta (GSK3β)' and 'Tau Tubulin Kinase 1 (TTBK1)' were selected targets for tau phosphorylation pathway.

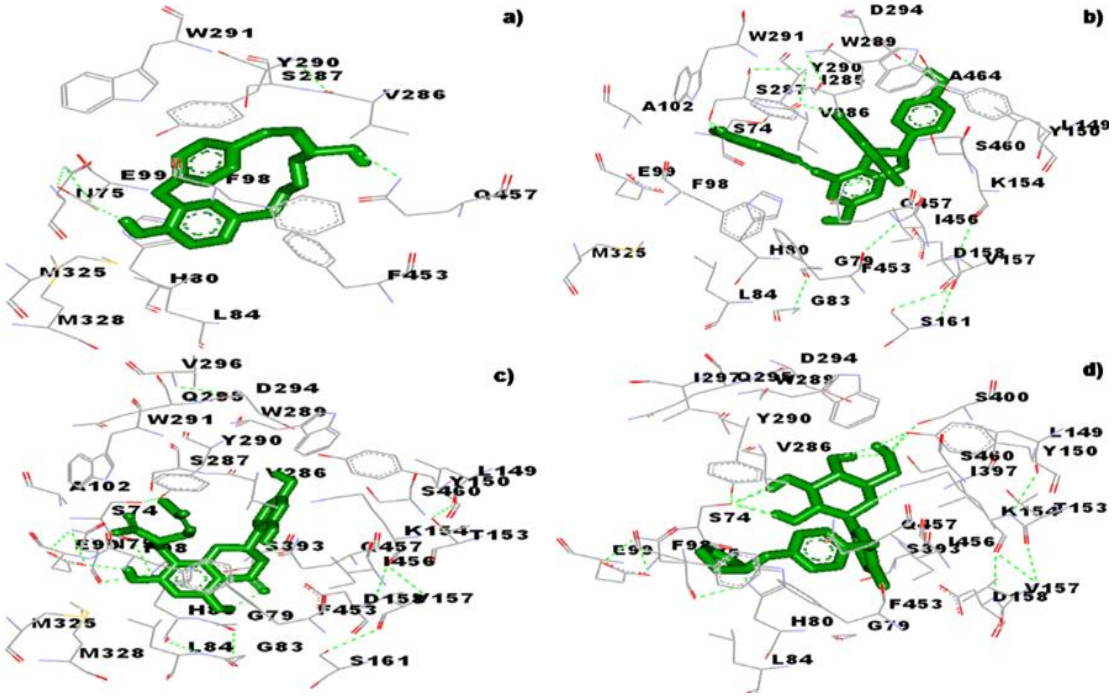

Fig. 1: Amino acids involved in 'Sodium glucose transport protein 2 (SGLT2)' interaction with natural Sodium-Glucose co-transporters 2 inhibitors and control. a) SGLT2-AcerogeninA interaction, b) SGLT2-ε viniferin interaction, c) SGLT2-SophoraflavanoneG interaction and d) SGLT2-Empagliflozin interaction. The ligands are shown in 'stick' representation.

Acerogenin A, ε-viniferin, and Sophoraflavanone G showed promising interaction with the active site of AChE (Table 4). ε-viniferin interaction with AChE was even better than control galantamine with ΔG and Ki values as '-8.92kcal/mol and 287.74nM' and '-8.69kcal/mol and 423.73nM', respectively. ΔG and Ki values for 'Acerogenin A-AChE' and 'Sophoraflavanone G-AChE' interaction were '-8.37kcal/mol and 734.75nM' and '-7.65kcal/mol and 2.45μM', respectively. Three amino acid residues, namely, S203, E334, and H447 are part of the AChE catalytic triad (Sussman et al., 1991; Remya et al.,2014). Importantly, among these three amino acids, Acerogenin A and Sophoraflavanone G showed interaction with two amino residues S203 and H447, while, ε-viniferin interacted with H447 amino acid residue only (Figure 2).

Table 4: Molecular docking results of 'Acetylcholinesterase (AChE)', 'β-secretase (BACE1)', 'Fyn Kinase (Fyn)', ' 'Glycogen synthase kinase 2 beta (GSK3β' and 'Tau Tubulin Kinase 1(TTBK1)' interaction with natural Sodium-Glucose co-transporter 2 (SGLT2) inhibitors and control compounds

Compounds	AChE		BACE1		Fyn		GSK3β		TTBK1	
	Binding Energy (ΔG) kcal/mol	Inhibition Constant (Ki)	Binding Energy (ΔG) kcal/mol	Inhibition Constant (Ki)	Binding Energy (ΔG) kcal/mol	Inhibition Constant (Ki)	Binding Energy (ΔG) kcal/mol	Inhibition Constant (Ki)	Binding Energy (ΔG) kcal/mol	Inhibition Constant (Ki)
Acerogenin A	-8.37	734.75nM	-7.93	1.53μM	-7.87	1.72μM	-7.67	2.37μM	-7.90	1.61μM
Alstiphyllanine D	-5.03	205.93μM	-2.96	6.82mM	-6.78	10.64μM	-6.07	35.27μM	-4.28	724.31μM
ε-viniferin	-8.92	287.74nM	-7.56	2.86μM	-8.75	387.80nM	-7.01	7.31μM	-7.60	2.69μM
Phlorizin	-2.88	7.71mM	-4.19	847.58μM	-5.70	66.91μM	-4.71	352.09μM	-4.18	860.86μM
Sophoraflavanone G	-7.65	2.45μM	-6.05	37.06μM	-5.61	77.73μM	-7.05	6.82μM	-5.99	40.67μM
Galantamine	-8.69	423.73nM	-	-	-	-	-	-	-	-
Verubecestat	-	-	-8.99	255.44nM	-	-	-	-	-	-
Leuco-methylthioninium	-	-	-	-	-6.33	23.10μM	-6.29	24.65μM	-6.11	33.46μM

On the other hand, AChE interaction with acetylcholine involves W86, E202, and Y337 amino acid residues (Kua et al., 2003), and interestingly, Acerogenin A, ε-viniferin, and Sophoraflavanone G all showed interaction with these three important amino acids.

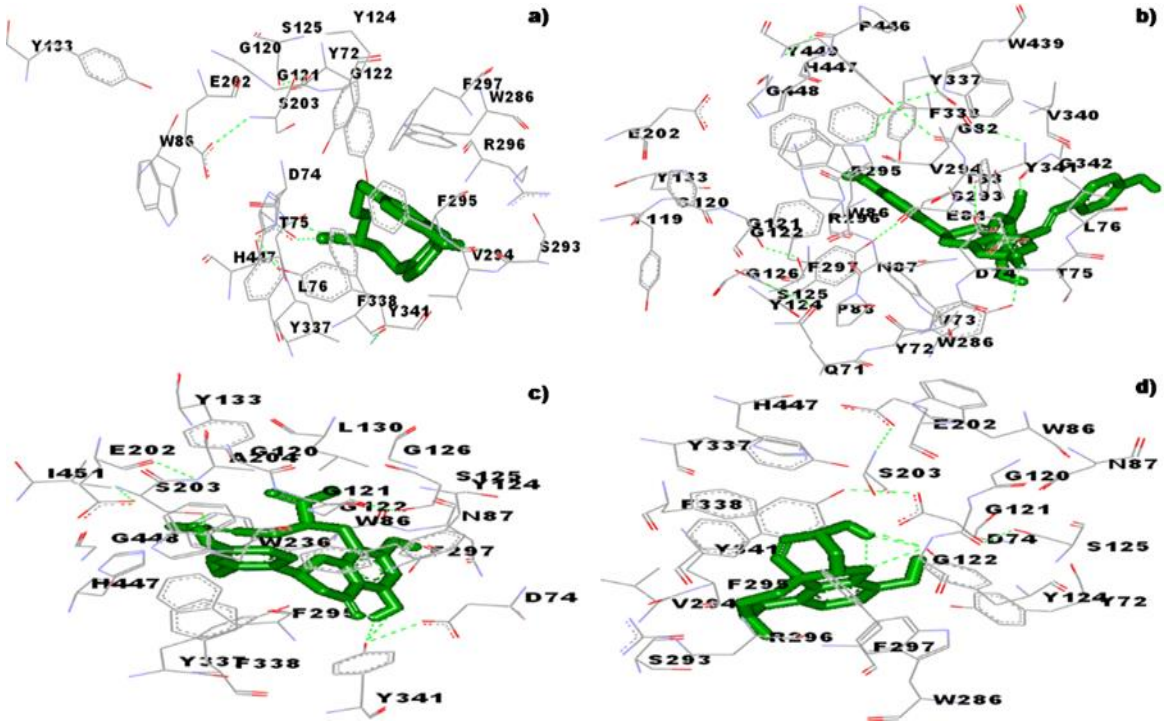

Fig. 2: Amino acids involved in 'Acetylcholinesterase (AChE)' interaction with natural Sodium-Glucose co-transporters 2 inhibitors and control. a) AChE-AcerogeninA interaction, b) AChE-ε viniferin interaction, c) AChE-SophoraflavanoneG interaction and d) AChE-Galantamine interaction. The ligands are shown in 'stick' representation.

Amyloidβ accumulation and generation in the brain via the amyloid precursor pathway is associated with an enzyme known as BACE-1. To avoid or reduce the brain amyloidβ load in AD patients, nowadays BACE-1 is considered a promising target (Ghosh et al., 2008). In the present study, none of the natural SGLT2 inhibitors have shown better interaction than positive control Verubecestat (Table 4). However, Acerogenin A, ε-

viniferin, and Sophoraflavanone G have shown somewhat hopeful interaction results against BACE-1 active site. ΔG and Ki values of 'Acerogenin A-BACE1', 'ε-viniferin-BACE1' and 'Sophoraflavanone G-BACE1' interactions were '-7.93kcal/mol and 1.53μM', '-7.56kcal/mol and 2.86μM' and '-6.05kcal/mol and 37.06μM', respectively. On the other hand, 'Verubecestat-BACE1' interaction ΔG and Ki values were -8.99kcal/mol and 255.44nM, respectively. Amino acid residues, L30, Y71, T72, Q73, K107, F108, W115, I118, G230, and T231 were found to be common in BACE-1 interaction with Acerogenin A, ε-viniferin, Sophoraflavanone G, and positive control Verubecestat (Figure 3).

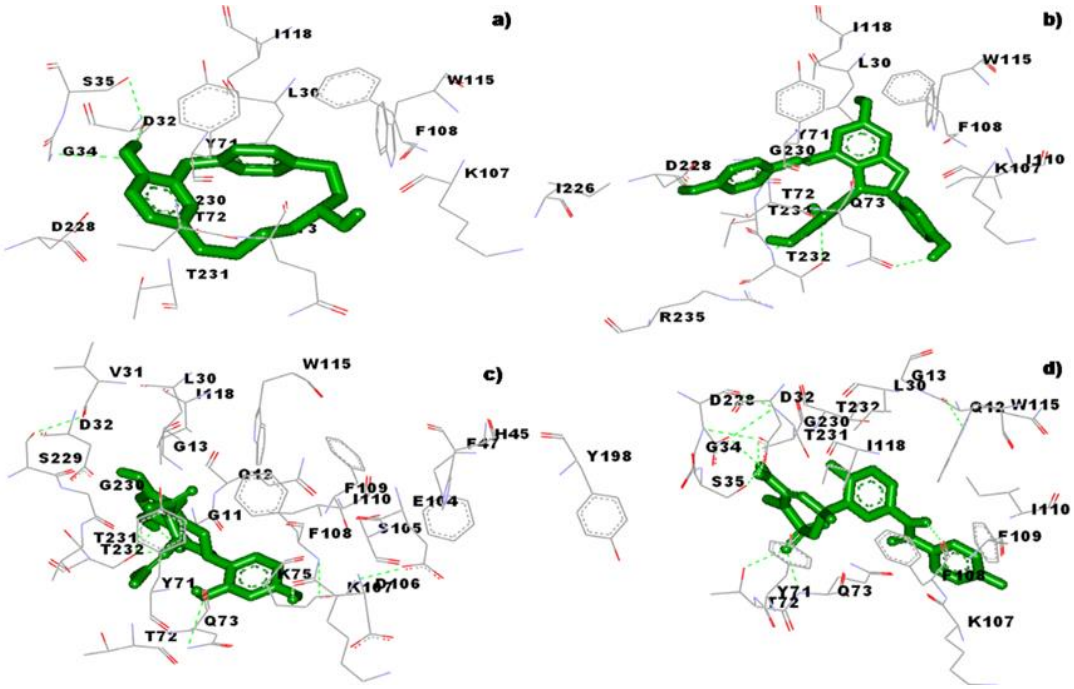

Fig. 3: Amino acids involved in '□secretase (BACE1)' interaction with natural Sodium-Glucose co-transporters 2 inhibitors and control. a) BACE1-AcerogeninA interaction, b) BACE1-ε viniferin interaction, c) BACE1-SophoraflavanoneG interaction and d) BACE1-Verubecestat interaction. The ligands are shown in 'stick' representation.

It has been observed that D32 and D228 play an important role in selective inhibition of BACE1 (Hernández-Rodríguez et al., 2016), in this study Acerogenin A and Verubecestat showed interaction with these two amino acid residues of BACE-1 active site. Also, Rajasekhar et al. (2015) have shown that the F108 side chain of the flap pocket has interacted eminently with the BACE-1 inhibitors. In this study, all the compounds interacted with the F108 amino acid residue of the BACE-1 active site. The binding energy of BACE-1 inhibition of natural SGLT2 inhibitors was less than the positive control, although, we tried to target the amyloidogenic pathway by another approach i.e., estimating amyloidβ anti-aggregation potential directly.

Amyloidβ (Aβ) peptide is a 39-42 amino acid residues long fragment produced by proteolytic cleavage of amyloid precursor protein with the help of BACE-1 and γ-secretase enzymes (Masters et al., 1985; Kang et al., 1987). Accumulation of these fragments results in amyloid plaque formation in the brain of AD patients. A more common form of Aβ peptide fragment that was observed in AD patient brain is 1-42 amino acid residues (Aβ1-42) long (Jarrett et al., 1993; Burdick et al., 1992; Riek et al., 2001). Deep structural insight of Aβ1-42 fragment showed that first 1 to 17 amino acid residues are not organized while 18 to 42 amino acids form a β-turn-β-fold motif with two parallel intermolecular β sheets (Lührs et al., 2005). At least two of these Aβ1-42 peptides are needed to form the reiterating structural base of protofilament. Interaction patterns of β-turn-β-fold motifs have elucidated the sequence selectivity and cooperativity for Aβ fibril development. In the present study, firstly β-turn-β-fold of Aβ1−42 peptide (Aβ17-42) were docked with each other with the help of the Hex 5.1 docking tool to decipher the specificity towards the respective motif and to comprehend the resultant conformational adjustments the beta-strand adopt in aggregate formation. Total interaction energy (E-total) for Aβ17-42 and Aβ17-42 interaction were found to be -1236.83 KJ/mol (Table 5). Afterward, we estimated the E-total of compound bounded Aβ17-42 with the native form of Aβ17-42 for each natural SGLT2 inhibitor to observe the changes in interaction pattern. Alstiphyllanine D, ε-viniferin and Sophoraflavanone G have significantly reduced the 'Aβ17-42' - 'Aβ17-42' interaction with E-total value as -766.58KJ/mol, -724.56KJ/mol and -740.14KJ/mol, respectively. These E-total values are very near to the E-total value of positive control curcumin i.e., -760.75KJ/mol. Recently, Bibi et al. (2019) have also applied a similar approach to

observe the inhibitory effect of an anticancer drug (bexarotene) on Aβ17-42 aggregation.

Microtubules are stabilized by a microtubule-associated protein known as tau, and tau hyperphosphorylation is a hallmark feature of AD (Weingarten et al., 1975; Avila et al., 2004; Alonso et al., 1996; Ballatore et al., 2007). There are more than 20 kinases that participate in phosphorylation of tau protein and the majority of these kinases are involved in phosphorylating AD sites of tau (Martin et al., 2013). Each of tau phosphorylating kinase has specific phosphorylation sites and contributes to neurodegeneration associated with AD. Tau hyperphosphorylation causes abnormal tau aggregation and diminishes its affinity towards microtubules, this may lead to disruption of microtubules mediated axonal growth, signal propagation, and vesicle transport (Gendron et al., 2009; LaPointe et al., 2009). These adverse effects might aggravate the symptoms of AD. In the present study, we have chosen three potential tau phosphorylation targets i.e., Fyn, GSK 3β, and TTBK1.

Table 5: Docking results of interaction of one β-turn-β-fold of Aβ1−42 peptide with another β-turn-β-fold of Aβ1−42 peptide before and after binding of natural SGLT2 inhibitors

Target	Ligand	E-total (KJ/mol)
β-turn-β-fold of Aβ1−42 peptide (Aβ17-42)	β-turn-β-fold of Aβ1−42 peptide (Aβ17-42)	-1236.83
Aβ17-42	Aβ17-42bounded with Acerogenin A	-1226.15
Aβ17-42	Aβ17-42bounded with Alstiphyllanine D	-766.58
Aβ17-42	Aβ17-42bounded with ε-viniferin	-724.56
Aβ17-42	Aβ17-42bounded with Phlorizin	-1058.28
Aβ17-42	Aβ17-42bounded with Sophoraflavanone G	-740.14
Aβ17-42	Aβ17-42bounded with Curcumin	-760.75

Fyn belongs to the Src family of non-receptor tyrosine kinases that directly associates with tau and phosphorylates tyrosine residues of tau protein. Its role in AD has been linked due to its participation in tau hyperphosphorylation. Thus, Fyn inhibition is considered a therapeutic intervention for AD (Nygaard et al., 2014; Nygaard et al., 2018). In this study, Acerogenin A, Alstiphyllanine D, and ε-viniferin showed better interaction with the active site domain of Fyn kinase enzyme when compared with positive control Leuco-methylthioninium. ΔG and Ki values for 'Acerogenin-Fyn', 'Alstiphyllanine D-Fyn', 'ε-viniferin-Fyn' and 'Leuco-methylthioninium-Fyn' interactions were found to be '-7.87kcal/mol and 1.72μM', '-6.78kcal/mol and 10.64μM', '-8.75kcal/mol and 387.80nM' and '-6.33kcal/mol and 23.10μM', respectively (Table 4). L17, V25, A37, K39, E54, T82, G88, L137, A147, and D148 were the important amino acid residues of 'kinase' domain of Fyn that were commonly interacting with Acerogenin A, Alstiphyllanine D, ε-viniferin and positive control Leuco-methylthioninium (Figure 4).

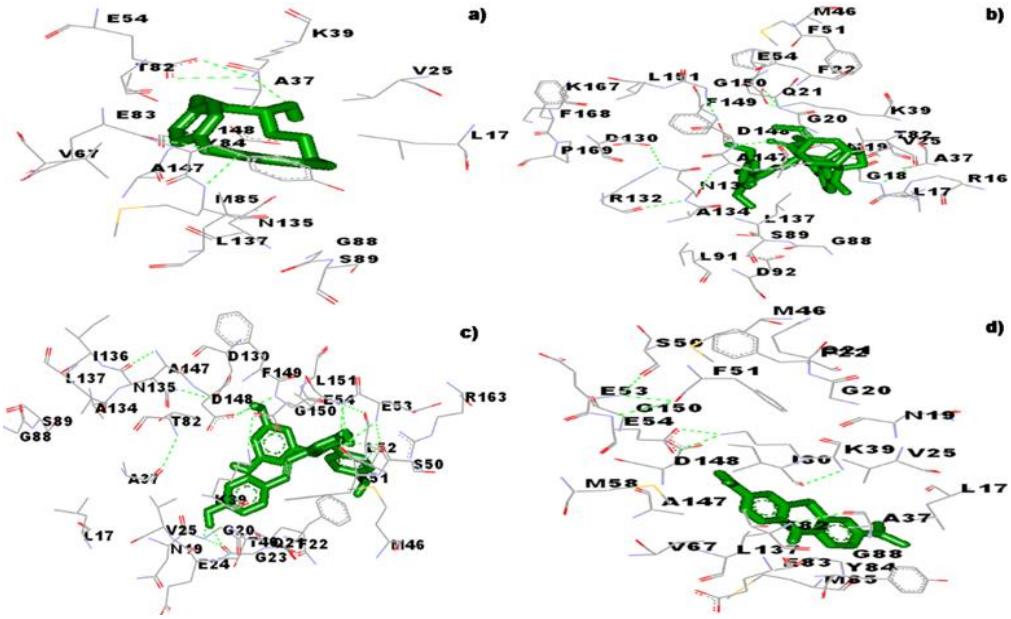

Fig. 4: Amino acids involved in 'Fyn kinase (Fyn)' interaction with natural Sodium-Glucose co-transporters 2 inhibitors and control. a) Fyn-AcerogeninA interaction, b) Fyn-AlstiphyllanineD interaction, c) Fyn-SophoraflavanoneG interaction and d) Fyn-Leucomethylthioninium interaction. The ligands are shown in 'stick' representation.

GSK3β is a serine/threonine-protein kinase, that plays a pivotal role in AD pathogenesis via activating various pathways including tau hyperphosphorylation (Hooper et al., 2008; Kremer et al., 2011; Avila et al., 2012). Several reports revealed that it is a part of common pathology in T2DM and AD (Gao et al., 2012). Thus, it is regarded as one of the key targets for both these linked diseases. In this study, Acerogenin A, ε-viniferin, and Sophoraflavanone G interacted well with the active site of GSK3β. Interestingly molecular interactions of these natural SGLT2 inhibitors with GSK3β were better than positive control Leuco-methylthioninium (Table 4). Acerogenin A, ε-viniferin, Sophoraflavanone G and Leuco-methylthioninium molecular interaction with the 'kinase' domain of GSK3β estimated 'ΔG and Ki' values as '-7.67kcal/mol and 2.37μM', '-7.01kcal/mol and 7.31μM', '-7.05kcal/mol and 6.82μM' and '-6.29kcal/mol and 24.65μM', respectively. Amino acid residues, namely, I62, V70, A83, K85, Y134, V135, R141, L188, and C199 of the kinase domain of GSK3β are commonly involved in the interaction with Acerogenin A, ε-viniferin, Sophoraflavanone G, and Leuco-methylthioninium (Figure 5).

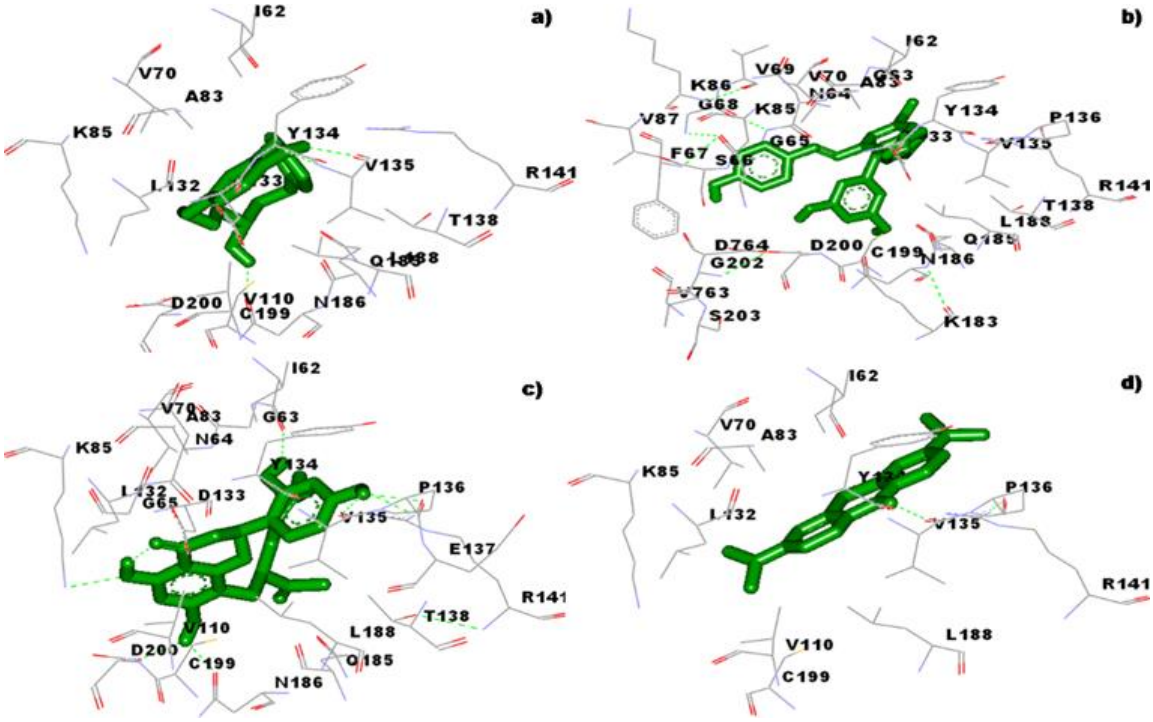

Fig. 5: Amino acids involved in 'Glycogen synthase kinase 2 beta (GSK3 GSK3β)' interaction with natural Sodium-Glucose co-transporters 2 inhibitors and control. a) GSK3 GSK3β -AcerogeninA interaction, b) GSK3 GSK3β- ε viniferin interaction, c) GSK3β-SophoraflavanoneG interaction and d) GSK3β-Leucomethylthioninium interaction. The ligands are shown in 'stick' representation.

Afreen et al. (2015) studied amino acid residues of GSK3β active site that was crucial for their selectivity over other kinases and found that interaction with D133, V135, and R141 was enough for active site inhibition and selectivity. Importantly, the selected natural SGLT2 inhibitors have shown interaction with V135 and R141 amino acid residues of GSK3β.

Also, it has been observed that disruption of hydration shell around V70 amino acid residue is also linked with increased activity of GSK3β inhibitors (Arfeen et al., 2015; Liang et al., 2016). In the present study, V70 amino acid residue of GSK3β active site was found to be interacting with Acerogenin A, ε-viniferin, Sophoraflavanone G, and Leuco-methylthioninium. TTBK1 is a neuron-specific kinase enzyme that plays an important role in tau hyperphosphorylation and tau aggregation (Sato et al., 2006). Thus, targeting TTBK1 is considered as a potential upcoming strategy against AD. In the present study, Acerogenin A and ε-viniferin were found to be better interacted with the ATP binding domain of TTBK1 as compared with positive control Leuco-methylthioninium (Table 4).

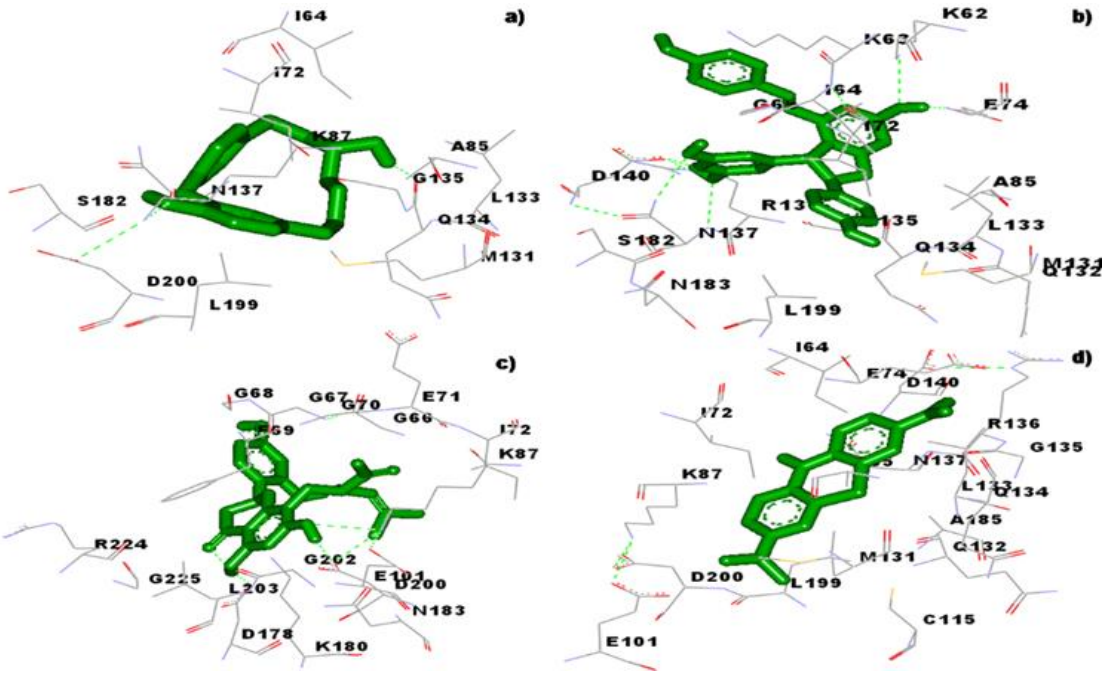

Fig. 6: Amino acids involved in 'Tau Tubulin Kinase 1 (TTBK1)' interaction with natural Sodium-Glucose co-transporters 2 inhibitors and control. a) TTBK1-AcerogeninA interaction, b) TTBK1- ε viniferin interaction, c) TTBK1-SophoraflavanoneG interaction and d) TTBK1-Leucomethylthioninium interaction. The ligands are shown in 'stick' representation.

The 'ΔG and Ki' values of 'Acerogenin A-TTBK1', 'ε-viniferin-TTBK1' and 'Leuco-methylthioninium-TTBK1' were estimated as '-7.90kcal/mol and 1.61μM', '-7.60kcal/mol, and 2.69μM' and '-6.11kcal/mol and 33.46μM', respectively. However, ' Sophoraflavanone G-TTBK1' interaction showed ΔG and Ki values as -5.99kcal/mol and 40.67μM, respectively. Amino acid residues, namely, I64, I72, A85, M131, L133, Q134, G135, N137, and L199 of the kinase domain of TTBK1 were found to be commonly interacting with Acerogenin A, ε-viniferin, and Leuco-methylthioninium (Figure 6). It can be safely stated from tau phosphorylation pathway inhibition results that Acerogenin A and ε-viniferin were the best among all-natural SGLT2 inhibitors.

On the whole, Acerogenin A, ε-viniferin, and Sophoraflavanone G were the best among all the natural SGLT2 inhibitors tested against AD targets. Acerogenin A is a cyclic diarylheptanoid isolated from *Acer nikoense* (Aceraceae) that is a potent SGLT2 inhibitor with 94μM IC50 against SGLT2 (Morita et al., 2010). ε-viniferin is a resveratrol dimer that belongs to the stilbenoids family, and the IC50 value of ε-viniferin against SGLT2 was estimated to be 110 μM (Oranje et al., 2019). On the other hand, Sophoraflavanone G is a phytoncide isolated from the *Sophora flavescens* (Fabaceae) family, and its IC50 value against SGLT2 was estimated to be 4.1 μM (Sato et al., 2007). In this study, these potent SGLT2 natural inhibitors were tested against various AD targets via *in silico* approach. They showed potential against cholinesterase, amyloidogenic, and tau phosphorylation pathways associated with AD. Further, *in vitro* and *in vivo* studies are needed to establish the dual therapeutic potential of these natural SGLT2 inhibitors. However, it has been observed that *in silico* findings often correlates well with wet-lab results. Nonetheless, the important structural insights of natural SGLT2 inhibitors binding to different AD targets were revealed and compared with positive control.

Conclusions

In the present study, anti-Alzheimer's potential for natural SGLT2 inhibitors was tested to predict their dual targeting efficacy. Natural SGLT2 inhibitors, namely, Acerogenin A, Alstiphyllanine D, ε-viniferin, Phlorizin, and Sophoraflavanone G were chosen for the study. Physicochemical properties, toxicity potential, and molecular interaction patterns against several targets of Alzheimer's Disease (AD) have been revealed. Findings suggested that Acerogenin A, ε-viniferin, and Sophoraflavanone G were the potent dual drug candidates for both Type 2 Diabetes Mellitus (T2DM) and AD. Acerogenin A and ε-viniferin both showed no violation of the Lipinski rule and no predicted toxicity. Acerogenin A, ε-viniferin, and Sophoraflavanone G all showed strong binding with the active site of AD therapeutic target enzymes. Interestingly, ε-viniferin and Sophoraflavanone G bind efficiently to the interface of β-sheets with the potential of converting neurotoxic amyloidβ aggregates to nontoxic forms. In the end, it could safely state that among the SGLT2 inhibitors tested ε-viniferin could provide a better scaffold to develop dual therapeutic agents against T2DM and AD shortly.

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Conflict of Interest

The author declares no conflict of interest related to this work.

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