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Original Research Article

Designing dual inhibitors for the treatment of Alzheimer's disease as well as Type 2 diabetes mellitus via pharmacoinformatics approach: A step towards better medication for diabetes-associated neurological disorder

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Abstract

Purpose: *To design dual inhibitors against Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) via pharmacoinformatics approach.*
Methods: *Dual Drug Candidates (DDC) were designed and explored for their molecular interaction with several AD and T2DM targets. Pterostilbene, a natural anti-T2DM compound was coupled with different cholinesterase inhibitors to design DDC. Orisis Datawarrior online property calculator tools, Autock 4.2 and Hex 5.1 were used to investigate the potency of all DDC relative to positive controls.*
Results: *The study found that DDC2 (pterostilbene - methylene linker -octa hydro amino phenothiazine), DDC3 (pterostilbene - ethylene linker - N-phthalimide) and DDC5 (pterostilbene - carbonyl linker - 2-methyl-4-aminoquinoline) were the most promising out of all the DDCs. DDC2 showed strong molecular interaction with most of the AD and T2DM targets, including acetylcholinesterase, butrylcholinesterase, β-secretase, receptor for advanced glycation end products and ATP sensitive potassium channel, dipeptidyl peptidase IV and sodium glucose transport protien 2. The findings also revealed the amyloid anti-aggregation potential of DDC.*
Conclusion: *The results show that DDC3 and DDC5 significantly interfer with the primary nucleation process of β amyloid. Thus, DDC2, DDC3 and DDC5 have strong anti-T2DM and anti-AD potential.*

Keywords: *Type 2 Diabetes Mellitus, Alzheimer's disease, Dual drug candidate, Amyloid-beta, Pterostilbene*

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INTRODUCTION

Alzheimer's disease (AD) and Type 2 diabetes mellitus (T2DM) are two of the major debilitating diseases sharing common pathophysiologies [1]. Indeed, T2DM is known to increase the risk of AD by 1.6 folds, and untreated T2DM patients develop AD a lot earlier than treated patients [2]. Unfortunately, the worldwide prevalence of both these disorders are increasing at an alarming

rate. According to World Alzheimer's Report 2018, 50 million of the world population are affected with dementia and AD, with the figures expected to increase progressively.

On the other hand, International Diabetes Federation 2017 statistics showed that 425 million individuals in the world are diabetic and the number might reach 629 million in the year 2045. In addition, every seven seconds a diabetic death occurs, and around 50 % of these deaths occur in an age group of below 60 years. Brain and pancreas are targeted organs for AD and T2DM, however, being anatomically and physiologically different they share some common pathophysiology. Clinical and animal model evidence have shown that islet amyloid polypeptide of pancreas could activate neuronal degradation and amyloid β peptide misfolding in AD [3]. It has been found that islet amyloid polypeptide enters the brain, links to amyloid β plaques and enhances misfolding of amyloid β.

Occurrence of AD is often associated with T2DM, thus, dual therapy targeting both the diseases could provide a novel alternative treatment approach. The present study deals with the design of some AD-T2DM dual therapeutic compounds and predict their efficacy against different AD and T2DM targets. Numerous natural compounds have been reported to be effective against T2DM, however, 'pterostilbene', a natural dimethylated resveratol analog was chosen for this study. Pterostilbene improves insulin sensitivity, reduces beta cell apoptosis and ameliorates diabetic nephropathy/retinopathy [5,6]. During this study, pterostilbene was coupled with different acetylcholinesterase inhibitors via linkers (Table S1). The coupled compounds were screened for physicochemical and toxicity profile before targeting them against AD. Alzheimer's disease is a complex disease with multiple pathways and hypotheses, in this study cholinergic and amyloidogenic pathways were selected.by targeting acetylcholinesterase, butrylcholine-sterase, beta-secretase, amyloid β aggregation and receptor for advanced glycation end products. Furthermore, to access the anti-T2DM potential the targets were ATP sensitive potassium channel, Dipeptidyl Peptidase IV, Sodium glucose transport protein 2.

EXPERIMENTAL

Preparation of dual drug candidates

The structures of the pterostilbene and different acetylcholinesterase inhibitors (Table S1) were retrieved from PubChem. The structures of the compounds were drawn in ChemDraw 8.0, coupled with methylene, ethylene and carbonyl linkers, and converted to their three-dimensional coordinates in Chem3D 8.0. Then they are subjected to structural optimization to avoid the structural strain through Merck Molecular Force Field (MMFF). Finally, all the compounds were saved in .pdb format for further docking studies.

Physicochemical and toxicity profile screening of dual drug candidates

Physico-chemical and toxicity profile of DDCs was done by Orisis Datawarrior property explorer tool (http://www.openmolecules.org/datawarrior/download.html). Various parameters, such as topological polar surface area (TPSA), molecular weight, cLogP, the number of hydrogen bond donors, the number of hydrogen bond acceptors, number of rotatable bonds and violations of Lipinski's rule of five [7] were calculated (Table 1). However, Zhao *et al* [8] method was used to calculate the percentage of absorption.

% of Absorption = 109 – (0.345 × TPSA)

Toxicity profile screening included mutagenicity, tumorogenicity, reproductive and irritability effects of DDC (Table 2).

Retrieval of target proteins and positive control drugs structures from databases

The three-dimensional structure of target proteins acetylcholinesterase (AChE) [ID: 3LII], butyrylcholinesterase (BChE) [ID: 1P0I], β-secretase (BACE-1) [ID: 1W51], β-turn-β-fold of $A\beta_{1-42}$ peptide ($A\beta_{17-42}$) [ID: 2BEG], receptor for advanced glycation end products (RAGE) [ID: 3CJJ], ATP sensitive potassium channel (KATP) [ID: 6BAA] and dipeptidyl peptidase IV (DPPIV) [ID: 2P8S] were retrieved from Protein Data Bank. However, three-dimensional structure of sodium glucose transport protein 2 was prepared by using Swiss Model Workspace after retrieving the amino acid sequence from Uniprot [P31639]. Control drugs tacrine [CID: 1935], AZD3293 [CID: 67979346], curcumin [CID: 969516], glimepiride [CID: 3476], Saxagliptin [CID: 11235729] and canagliflozin [CID: 24812758] were obtained from PubChem database.

Molecular docking

Autodock 4.2 was used to dock the ligands with protein following the protocol of Rizvi *et al* [9]. Energy minimization of each ligand was done by MMFF94 force field and gasteiger partial charges were added. Rotatable bonds were defined after

including non-polar hydrogen atoms. Kollman united atom type charges, Solvation parameters and hydrogen atoms were added with the help of AutoDock. 'Auto Grid' was used to set the dimensions of grid (60 x 60 x 60 Å with 0.375 Å point separation). The x, y and z target coordinate values were kept as 90.81, 83.98 and -8.04 for AChE; 141.21, 115.41 and 40.40 for BChE; 73.79, 54.27 and 11.51 for BACE-1 and 54.56, -11.16 and 14.39 for RAGE. For KATP, DPPIV and SGLT2, different docking experiments were performed using known amino acid residues as target site. Van der Waals forces and the electrostatic interactions were estimated using AutoDock. 'Solis and Wets local search method' and 'Lamarckian genetic algorithm' were used to perform docking simulation. One hundred runs were performed for each docking trials with endpoint set to 2,500,000 energy evaluations. The population size was set at 150.

The anti-aggregation potential of DDC compounds was studied by Hex 5.1. Docking of DDC bound $A\beta_{17-42}$ peptide with unbound $A\beta_{17-42}$ peptide was performed based on 'shape only' correlation type, first fourier transform mode as 3D Fast Lite, grid dimension as 0.75 and rest all parameters were kept as default. The figure for the results generated in docking experiments were elucidated using Discovery Studio 2.5 (Accelrys).

RESULTS

In the present study, a natural diabetic compound 'pterostilbene' was coupled with different acetylcholinesterase (AChE) inhibitors to design five dual targeting compounds and named them as Dual Drug Candidates (DDC) (Table S1). The physicochemical properties and toxicity potential of DDC1 to DDC5 and control compounds/drugs were tested. Table 1 shows all the physicochemical properties and Lipinski violations and percentage of absorption. According to Lipinski rule [7], DDC1, DDC4 and DDC5 showed only one violation in cLogP value (logarithm of compound partition coefficient between *n*-octanol and water) while DDC2 and DDC3 showed no violation that is similar to the control compounds (Table 1). Table 2 represents the toxicological potential of DDC, among all DDCs, DDC1, DDC4 and DDC5 showed high mutagenic effect and reproductive effect whereas DDC2 and DDC3 showed only reproductive effect.

The results for cholinesterase interaction with DDCs are presented in Table 3. All DDCs appear to be promising inhibitors of AChE when compared to Tacrine. However, DDC1, DDC2, DDC4 and DDC5 appear to be most potent against AChE with binding energy (∆G) and inhibition constant (Ki) of '-11.33kcal/mol and 4.94 nM', '-11.07kcal/mol and 7.71nM', '-11.55kcal/mol and 3.42nM' and '-11.23kcal/mol and 5.84nM', respectively. Interestingly, DDC2, DDC3, DDC4 and DDC5 effectively bound to two amino acid residues, namely, S203 and H447 of the catalytic triad of AChE (Figure 1). Concurrently, compounds DDC1 to DDC5 all showed better binding with butyrylcholinesterase (BChE) than tacrine. Among them, DDC1, DDC2 and DDC5 were the best with ∆G and ki values of '-8.77kcal/mol and 0.37μM', '-9.52kcal/mol and 0.10μM' and '-8.55kcal/mol and 0.53μM', respectively. Interestingly, DDC1, DDC4 and DDC5 showed interaction with F329 of BChE peripheral site (Figure 2).

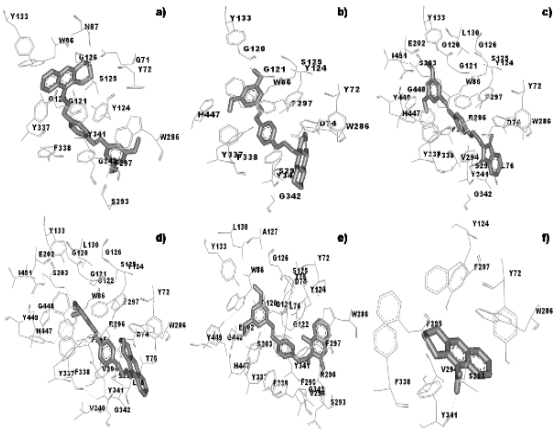

Figure 1: Amino acids involved in 'acetylcholinesterase (AChE) interaction with dual drug candidates. a) AChE-DDC1 interaction, b) AChE-DDC2 interaction, c) AChE-DDC3 interaction, (d) AChE-DDC4 interaction; (e) AChE-DDC5 interaction and (f) AChE-Tacrine interaction. The ligands are shown in 'stick' representation

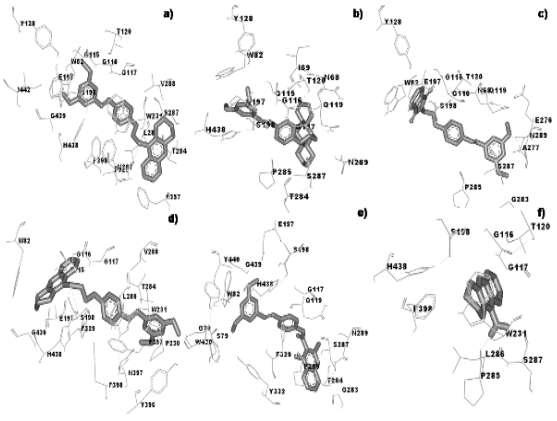

Figure 2: Amino acids involved in Butyrylcholinesterase (BChE) interaction with dual drug candidates. (a) BChE-DDC1 interaction, b)

BChE-DDC2 interaction, (c) BChE-DDC3 interaction; (d) BChE-DDC4 interaction; (e) BChE-DDC5 interaction and (f) BChE-Tacrine interaction. The ligands are shown in 'stick' representation.

β-secretase (BACE-1) molecular docking study showed that only two compounds DDC2 and DDC3 appear to be promising when compared with known BACE-1 inhibitor AZD3293 in terms of binding energy. The results of ∆G and Ki for DDC2, DDC3 and AZD3293 against BACE-1 were estimated to be '-7.95 kcal/mol and 1.49μM', '-8.07 kcal/mol and 1.22 μM' and '-7.97 kcal/mol and 1.43 μM', respectively (Table 4).

Table 1: Physicochemical properties of dual drug candidates and control compounds

Compound	Physiochemical parameter							
	Absorption** **(%)**	**Topological polar surface Area (Å)²**	**Mol. Weight**	**cLogP*****	**H bond donors**	**H bond acceptors**	**Number of rotat. bonds**	**Lipinski's violation**
Rule	**-**	**-**	**<500**	**≤5**	**<5**	**<10**	**≤10**	**≤1**
DDC 1	90.84	52.61	466.58	6.34	1	5	8	1
DDC 2	85.45	68.26	490.66	4.54	1	5	8	0
DDC 3	86.55	65.07	429.47	4.48	0	6	8	0
DDC 4	90.84	52.61	480.60	6.32	1	5	9	1
DDC 5	80.13	83.67	440.49	5.19	1	6	7	1
Tacrine*	95.57	38.91	198.26	2.50	1	2	0	0
AZD3293*	83.86	72.86	412.53	3.45	1	5	3	0
Curcumin*	76.89	93.06	368.38	2.94	2	6	8	0
Glimepiride*	63.09	133.06	490.62	3.51	3	9	6	0
Miglitol*	72.89	104.39	207.22	-2.68	5	6	3	0
Saxagliptin*	77.82	90.35	315.41	0.349	2	5	2	0
Canagliflozin*	68.15	118.39	444.52	3.27	4	5	5	0

*Control drugs/compounds; **Percentage of Absorption (% of Absorption) was calculated viz: % of Absorption= 109 – [0.345 ×; pological Polar Surface Area]; ***Logarithm of compound partition coefficient between *n*-octanol and water

Table 2: Toxicity potential of dual drug candidates and control compounds

Compound	Toxicity risk			
	Mutagenic	***Tumorigenic***	***Reproductive effect***	***Irritant***
DDC 1	High	None	High	None
DDC 2	None	None	High	None
DDC 3	None	None	High	None
DDC 4	High	None	High	None
DDC 5	High	None	High	None
Tacrine	High	High	None	None
AZD3293	None	None	None	None
Curcumin	None	None	None	None
Glimepiride	None	None	None	None
Miglitol	None	None	None	None
Saxagliptin	None	None	None	None
Canagliflozin	None	None	None	None

Table 3: Molecular docking results of cholinesterase interaction with dual drug candidates

Compound	Acetylcholinesterase		Butyrylcholinesterase	
	Binding energy (ΔG) kcal/mole	***Inhibition constant (Ki)***	***Binding Energy (ΔG) kcal/mole***	***Inhibition Constant (Ki) μM***
DDC 1	-11.33	4.94nM	-8.77	0.37
DDC 2	-11.07	7.71nM	-9.52	0.10
DDC 3	-9.78	68.14nM	-7.88	1.67
DDC 4	-11.55	3.42nM	-6.97	7.73
DDC 5	-11.23	5.84nM	-8.55	0.53
Tacrine	-6.42	19.75μM	-6.35	22.07

Table 4: Amino acid residues involved in 'β-secretase (BACE1) and dual drug candidates' interactions

Compound	Binding energy (ΔG) kcal/mol	Inhibition constant (Ki)μM)	Interacting amino acids
DDC 1	-6.92	8.51	Q12, G13, L30, F47, Q73, D106, K107, F108, F109, I110, N111, I118, G230, T231, T232
DDC 2	-7.95	1.49	Y68, T72, Q73, G74, K75, D106, K107, F108, I110
DDC 3	-8.07	1.22	A43, P44, H45, F47, T103, E104, S105, D106, K107, F108, F109, N111
DDC 4	-5.01	212.47	G11, Q12, G13, L30, Q73, G74, K75, D106, K107, F108, I110, I118, G230, T232
DDC 5	-7.04	6.88	H45, F47, Y71, T72, Q73, K107, F108, F109, I110, W115, G230, T231
AZD3293	-7.97	1.43	H45, F47, S105, D106, K107, F108, F109, I110, N111

Initially, the unbound form of β-turn-β-fold of $Aβ_{1-42}$ peptide ($Aβ_{17-42}$) were docked with each other to reveal the confirmation adjustment and specificity towards the respective motif to form amyloid aggregates with the help of Hex 5.1. Interaction of $Aβ_{17-42}$ with $Aβ_{17-42}$ showed a total interaction energy (E-total) as -1038.18 kJ/mol with 21 hydrogen bonds involved in the interaction (Table 5). The interaction of compound bound form of $Aβ_{17-42}$ with the native form of $Aβ_{17-42}$ were checked for each DDC to visualize the shift in the interaction pattern. Furthermore, it was found that DDC3 bounded $Aβ_{17-42}$ interacted the native form of $Aβ_{17-42}$ with an E-total of -698.58 KJ/mol, and only 6 hydrogen bonds were involved. However, DD5 bounded $Aβ_{17-42}$ interacted the native form of $Aβ_{17-42}$ with an E-total of -732.50 KJ/mol, and 14 hydrogen bonds were involved.

'Receptor for Advanced Glycation End products (RAGE)-DDCs' molecular interaction results are presented in Table 6. Among all DDCs, DDC2, DDC3 and DDC5 showed better interaction with the domain 1 of RAGE i.e., almost equivalent to the control curcumin, whereas, P42, P46, Q47, R48, M102, N103, R104 were the common amino acid residues of RAGE that showed interaction with DDC 2, DDC 3, DDC 5 and curcumin. Hence, it could be concluded that DDC2, DDC3 and DDC5 were the best candidates for amyloid aggregation inhibition. However, to check the anti-T2DM potential, the interaction potential of test compounds were checked with ATP sensitive potassium channel (KATP Channel), dipeptidyl peptidase IV (DPP IV) and sodium glucose transport protein 2 (SGLT2).

Table 5: Docking results of interaction of one β-turn-β-fold of $Aβ_{1-42}$ peptide ($Aβ_{17-42}$) with another β-turn-β-fold of $Aβ_{1-42}$ ($Aβ_{17-42}$) peptide before and after binding of dual drug candidates

Target	Ligand	E-total (KJ/mol)	No. of H-bonds
$Aβ_{17-42}$	$Aβ_{17-42}$	-1038.18	21
$Aβ_{17-42}$	$Aβ_{17-42}$ bounded with DDC 1	-870.51	17
$Aβ_{17-42}$	$Aβ_{17-42}$ bounded with DDC 2	-866.77	16
$Aβ_{17-42}$	$Aβ_{17-42}$ bounded with DDC 3	-698.58	6
$Aβ_{17-42}$	$Aβ_{17-42}$ bounded with DDC 4	-804.54	15
$Aβ_{17-42}$	$Aβ_{17-42}$ bounded with DDC 5	-732.50	14
$Aβ_{17-42}$	$Aβ_{17-42}$ bounded with Curcumin	-967.93	21

Table 6: Amino acid residues involved in RAGE and dual drug candidate interactions

Compound	Binding Energy (ΔG) kcal/mol	Inhibition Constant (Ki)μM	Interacting amino acids
DDC 1	-6.83	9.81	P46, Q47, R48, L49, E50, S65, P66, R104, N105
DDC 2	-7.25	4.85	P42, P46, Q47, R48, M102, N103, R104, N105, G106, K107
DDC 3	-7.06	6.68	P42, K44, P45, P46, Q47, R48, M102, N103, R104, G106
DDC 4	- 6.86	9.44	P46, Q47, R48, L49, S65, P66, R104, N105
DDC 5	-7.11	6.11	P42, P46, Q47, R48, L49, E50, S65, P66, M102, N103, R104
Curcumin	-7.07	6.62	P42, P46, Q47, R48, L49, E50, S65, M102, N103, R104, N105

Table 7: Docking results for interaction of 'DDCs' with KATP Channel', 'DPP IV' and SGLT2'

Compound	KATP channel		DPP IV		SGLT2	
	Binding energy (ΔG) kcal/mol	***Inhibition constant (Ki) μM***	***Binding energy (ΔG) kcal/mol***	***Inhibition constant (Ki) μM***	***Binding Energy (ΔG) kcal/mol***	***Inhibition constant (Ki) μM***
DDC 1	-7.61	2.65	-6.10	33.72	-8.32	0.79
DDC 2	-8.33	0.78	-6.58	15.06	-8.29	0.83
DDC 3	-7.45	3.45	-5.71	65.41	-7.12	6.04
DDC 4	-7.39	3.81	-6.08	35.12	-7.60	2.67
DDC 5	-6.81	10.23	-6.05	36.97	-8.22	0.94
ride	-6.93	8.31	-7.25	4.86	-7.23	5.04

In this study, DDC1, DDC2, DDC3, DDC4 showed better binding with KATP than to control glimepiride (Table 7). ΔG and Ki values for DDC1, DDC2, DDC3, DDC4 and glimepiride interaction with KATP were found to be '-7.61kcal/mol and 2.65 μM', '-8.33kcal/mol and 0.78 μM', '-7.45kcal/mol and 3.45 μM', '-7.39kcal/mol and 3.81 μM' and '-6.93kcal/mol and 8.31 μM', respectively. It could be depicted from the results (Figure 3) H175, I296, T297 were the commonly interacting amino acid residues of KATP channel with DDC1, DDC2, DDC3 and DDC4 as well as the positive control glimepiride.

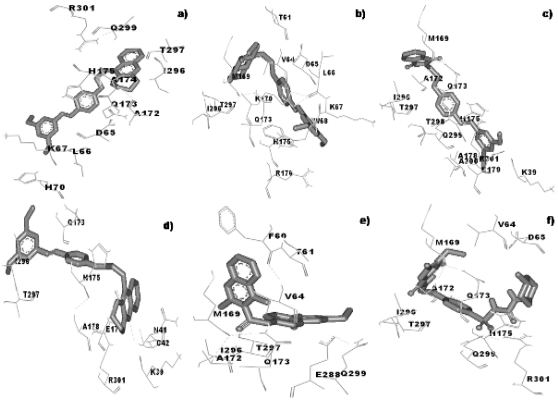

Figure 3: Amino acids involved in 'KATP' interaction with 'Dual drug candidates'. a) KATP-DDC1 interaction, b) KATP-DDC2 interaction; (c) KATP-DDC3 interaction; (d) KATP-DDC4 interaction, e) KATP-DDC5 interaction, and (f) KATP- Glimepiride interaction. The ligands are shown in 'stick' representation

During DPPIV-DDCs interaction studies, the tested compounds were compared with a known selective DPPIV inhibitor saxagliptin. Unfortunately, none of the tested compounds, DDC1 to DDC5 showed better interactions than the positive control saxagliptin (Table 7). However, 'DDC2-DPPIV interaction' appears to be somewhat promising with ΔG and Ki values of '-6.58 kcal/mol and 15.06 μM'. Figure 4 shows the interacting amino acids of DPPIV active site interacting amino acid results.

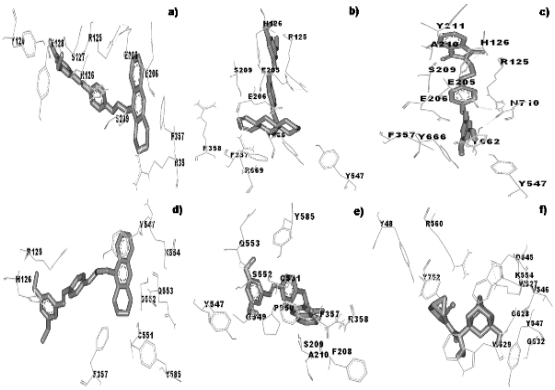

Figure 4: Amino acids involved in (DPPIV)' interaction with dual drug candidates'. a) DPPIV-DDC1 interaction; (b) DPPIV-DDC2 interaction; (c) DPPIV-DDC3 interaction; (d) DPPIV-DDC4 interaction; (e) DPPIV-DDC5 interaction and (f) DPPIV-Glimepiride interaction. The ligands are shown in 'stick' representation

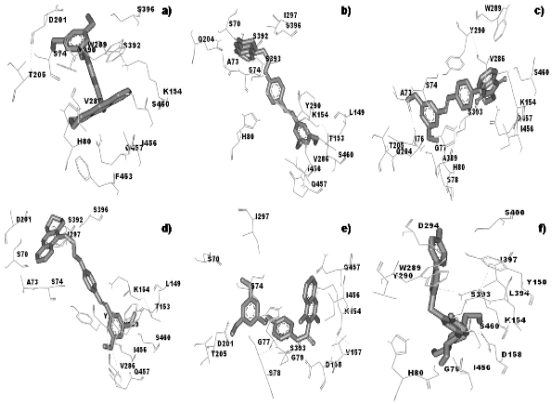

Figure 5: Amino acids involved in SGLT2 interaction with dual drug candidates. (a) SGLT2-DDC1 interaction; (b) SGLT2-DDC2 interaction, (c) SGLT2-DDC3 interaction, (d) SGLT2-DDC4 interaction, (e) SGLT2-DDC5 interaction and (f) SGLT2-Glimepiride interaction. The ligands are shown in 'stick' representation

The results from SGLT2-DDCs' interaction showed that DDC1, DDC2, DDC4 and DDC5 interacted with SGLT2 better than a positive control canagliflozin in terms of binding energy

(Table 7). ∆G and Ki values of DDC1, DDC2, DDC4 and DDC5 interaction with SGLT2 were '-8.32 kcal/mol and 0.79 μM', '-8.29 kcal/mol and 0.83 μM', '-7.60 kcal/mol and 2.67 μM' and '-8.22 kcal/mol and 0.94 μM', respectively. In addition, all the tested compounds showed interaction with the most important amino acid residue Q457 of SGLT2 (Figure 5).

DISCUSSION

Alzheimer's disease (AD) is a complex disease with a strong Type 2 Diabetes Mellitus (T2DM) linkage [2,10]. Interestingly, augmented amyloid plaque accumulation has been observed in the hippocampus of T2DM patients during the autopsy [11]. In addition, T2DM untreated patients have 1.6-fold more chances of developing AD than [2,10]. Till date, available treatment of AD such as use of acetylcholinesterase inhibitors and N-methyl D-aspartate receptor antagonist provides only the symptomatic relief.

However, a total of 132 agents are currently in clinical trials for AD treatment [12]. Unfortunately, in the past, a high failure rate has been observed in AD drug development [12]. The delay in treatment results in poor clinical response in AD patients. Hence early treatment in patients even at pre-clinical stages of AD is recommended. Phase III clinical trials have so far not approved any drug for dual drug therapy thus prompting the present study to design dual targeting compounds that could be plausibly used for the treatment of both linked diseases. To achieve this, we have coupled a natural diabetic compound pterostilbene [5,6] with different acetylcholinesterase (AChE) inhibitors to design five dual targeting compounds and named them Dual Drug Candidates (DDCs).

The major objective was to predict the anti-AD and anti-T2DM potential of DDCs. AD is a complicated disease with tau phosphorylation, cholinergic and amyloidogenic mechanism hypothesis. Out of these three, the cholinergic and amyloidogenic pathways were targeted in the study. Acetylcholinesterase and butrylcholinesterase (BChE) were targets for cholinergic pathways, and β-secretase (BACE1), Beta amyloid aggregation and (RAGE were targets for amyloidogenic pathway.

The amino acid residues, W86, E202 and Y337 were found to be involved in binding of acetylcholine to AChE [13]. In this study, DDC2, DDC3, DDC4 and DDC5 showed binding with all the three amino acid residues (W86, E202 and Y337). The catalytic triad of BChE is made-up of S198, E325 and H438 [14]. Out of all DDCs, DDC1, DD2, DDC4, DD5 and positive control tacrine interacted with S198 and H438. It has been reported that D70, F329 and Y332 are important amino acid residues of the peripheral site of BChE [15]. The compounds, DDC1, DDC4 and DDC5 interacted with F329 amino acid residue of BChE peripheral site. The above interaction studies concluded that DDC1 and DDC2 were the best among the five and could be explored further as dual drug candidates.

BACE-1 is involved amyloid precursor pathway and plays an important role in the generation and accumulation of amyloid β in brain. Amino acid residues, D106, K107 and F108 of BACE-1 active site were found to be commonly interacting with DDC2, DDC3 and AZD3293. Similarly, Hassan *et al* [16] observed strong hydrogen bonding of AZ3293 with K107. In another study, Rajasekhar *et al* [17] observed that BACE-1 inhibitors interacted with F108 amino acid of BACE-1.

Interestingly, it has been observed that Aβ peptide fragment of 1-42 amino acid residues ($A\beta_{1-42}$) is more dominant in AD patients than 1-40 amino acid residues Aβ ($A\beta_{1-40}$) peptide [18]. Lührs *et al* [18] have deeply studied the 3D structure of $A\beta_{1-42}$ fragment and found that β-turn-β-fold motif are formed from 18-42 amino acid residues while 1-17 amino acids are disordered. The protofilament is formed from parallel intermolecular β sheets of β-turn-β-fold motif of $A\beta_{1-42}$ peptide. Further these interactions of β-turn-β-fold motifs have shown the sequence cooperativity and selectivity for the Aβ fibril formation. In this study, DDC3 and DDC5 when bound to $A\beta_{17-42}$ markedly reduced the level of interaction with other native form of $A\beta_{17-42}$. Similar inhibitory results were obtained by Bibi *et al* [19] for anticancer drug (bexarotene).

Receptor for advanced glycation end products is immunoglobulin superfamily member that could bind to variety of ligands including Aβ peptides. In fact, RAGE interaction with Aβ peptides is responsible for the influx of circulating Aβ peptides in the brain [20]. It has been observed that up-regulation of RAGE leads to higher accumulation of Aβ peptides in the brain. In another report, augmented levels of RAGE were observed in the AD hippocampus [21]. Therefore, targeting RAGE would have a positive effect on AD treatment [20]. Receptor for Advanced Glycation End products crystal structure has two immunoglobulin domains, where 23 to 118 amino acid residues form domain 1 and 121-231 amino acid residues forms domain 2 that are linked via a short linker. Bibi *et al* [19] observed that

domain 1 of RAGE was critical for the binding of Aβ peptides and transportation across the blood brain barrier. Positively charged surface of RAGE is constructed by R29, K37, K39, K43, K44, R48, K52, R98, R104, K107, K110, R114 and R116 of domain 1 and R216 of domain 2 [22]. In this study, interestingly, DDC2, DDC3 and DDC5 showed better interaction with the domain 1 of RAGE.

ATP sensitive potassium channel (KATP Channel) channels have a major role in glucose triggered insulin secretion of pancreatic β cells. Closure of KATP channel initiates the secretion of insulin, while, opening of KATP channel results in vice versa [23]. KATP channel of pancreatic β cells is an octameric complex with 4 sulfonylurea-receptor regulatory subunits (SUR1) and 4 inward rectifying K channel subunits (Kir6). Sulfonylurea-receptor regulatory subunits 1 and Kir6 are important for functioning of KATP channels; Mg-ADP/ATP interact with SUR1 to stimulate the KATP channel activity, while ATP interact with Kir6 to close KATP channel. In this study, DDC1-4 showed better interaction than glimepiride.

Dipeptidyl Peptidase IV (DPP IV) is responsible for inactivation of incretin hormones glucose-dependent insulinotrophic polypeptide and glucagon like peptide-1. These incretin hormones act as triggers for secretion of insulin and regulation of blood glucose level. Thus, targeting DPP IV provides an alternative way to curb T2DM [24]. A comparative analysis of DDCs with a known DPPIV inhibitor Saxagliptin were performed in this study, however, none of the DDCs showed better interaction than saxagliptin.

Proximal convoluted tubules of kidneys have SGLT2 proteins which is responsible for maximum reabsorption of glucose [25]. Therefore, SGLT2 is considered as a newer target for T2DM and their inhibitors have been recently approved by FDA for T2DM treatment [25]. Investigation have shown that Q457 of SGLT2 is responsible for glucose reabsorption [26] and all our compounds interacted with Q457.

Overall, DDC2, DDC3 and DDC5 were the most promising among all dual drug candidates. However, it can be safely stated that DDC2 appears to be the best with potent affinity against most of the targeted proteins. Interestingly, DDC2 showed no violation of Lipinski rule, only reproductive toxicity and strong molecular interaction with cholinesterase, BACE-1, RAGE, KATP channel, DPP IV and SGLT2. The compound DDC2 has been designed on phenothiazine scaffold and coupled with pterostilbene. Phenothiazine is known for its cholinesterase inhibition potential and pterostilbene is an amazing natural anti-diabetic compound. Thus, combining these might provide an interesting dual drug candidate for future AD and T2DM therapy. Nevertheless, some important structural insights critical for binding of dual drug candidates with different AD and T2DM targets were revealed in this study. Parallel molecular docking experiments with positive control for each target helped to get nice comparative analysis on binding behavior of each dual drug candidate.

CONCLUSION

In the present study, five dual drug candidates (DDC) have been successfully designed and their physicochemical and toxicity profile evaluated. Extensive structural and interaction analysis predicts that DDC2, DDC3 and DDC5 are the most promising candidates. However, strong molecular interaction against most AD and all T2DM targets has been observed for DDC2. Thus, DDC2 should be further studied for dual drug therapy. Nonetheless, the results obtained in the present study may reduce the time and cost for the development of drugs against T2DM associated neurological disorders.

DECLARATIONS

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Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. TH and GS participated in physicochemical properties evaluation, assessment of toxicity potential, anti-Alzheimer's potential via targeting cholinergic and amyloidogenic pathways, data analysis and compilation of data. SMDR participated in designing of new compounds, assessment of amyloid-beta peptide clearance potential, anti-diabetic potential assessment and writing of manuscript. ASA and AM participated in revision, editing and proof reading of the final manuscript.

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