IN SILICO ASSESSMENT OF TYPE 2 DIABETES MELLITUS DRUGS AGAINST AMYLOID-B AND TAU AGGREGATION-FORMING SEGMENTS (KLVFFA, VQIVYK, GGVVIA, VQIINK) FOR ALZHEIMER'S DISEASE

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Abstract

This study reports in silico studies on approved type 2 diabetes mellitus (T2DM) drugs to combat aggregation-forming segments of tau and amyloid-β proteins in Alzheimer's disease (AD). The segments include 16-VQIVYK-21, 37-KLVFFA-42, 275-GGVVIA-280, 306-VQIINK-311. Ligand library includes only approved thirty-one small molecule species whose molecular weight is lower than 500 g/mol. The study aims to find one or more common small molecule inhibitors for four targets by adopting a multi-target drug design strategy. In this study, EGCG (Epigallocatechin Gallate) has been chosen as a positive control compound compared to the T2DM drugs. According to molecular docking results, common for four targets, five lead drugs for drug repurposing are suggested further experimental examination with combination or single-drug therapy methods. Lead molecules have higher docking scores from EGCG. In the determined five drugs, which are linagliptin, glimepiride, teneligliptin, canagliflozin, and glipizide, linagliptin has the highest docking score and so is advised as the most potent inhibitor candidate of tau and amyloid-β fibrils.

Keywords: *Molecular docking, Tau aggregation, Amyloid-β, Alzheimer's disease, Type 2 diabetes mellitus, Drug repurposing*

1. INTRODUCTION

A multi-target term means that a drug has affinity for more than one target [7]. Multi-target directed ligands (MTDLs) are treated as the concept of "one molecule, multiple targets" [1]. Polypharmacology refers to the use of drugs with multiple targets and is considered particularly useful in the treatment of multifactorial diseases such as Alzheimer's disease [2]. The use of compounds that have a complementary effect on two or more targets has attracted considerable attention at AD [3], and in this context, MTDLs for AD have been studied by many researchers [4-8]. In tau and amyloid-β proteins, some fibrils were found to be pioneer sites in aggregation formation through the formation of β-sheets and tau fibrils. Therefore, targeting these regions could be an effective treatment strategy to block the oligomerization of amyloid and tau at the beginning of the aggregation process. The hexapeptide fibrils known to drive aggregation in amyloid-β and tau proteins are 37-GGVVIA-42 and 16-KLVFFA-21 [9-11], 275-VQIINK-280 and 306-VQIVYK-311 [12-15]. T2DM is a risk factor for AD, and both diseases affect many people in developed countries [16]. Many common features have been found between T2DM and AD, such as oxidative stress, inflammation, dyslipidemia, and hypercholesterolemia [17]. Antidiabetic treatments have several neuroprotective effects and thus may also be useful in the treatment of AD [18, 19]. In this study, the key segments of aggregation were targeted and docking experiments were performed with thirty-one approved T2DM drugs. To efficiently evaluate the obtained results, a reference, epigallocatechin gallate (EGCG), was used as a positive control and standard compound. EGCG is a biologically active major component of green tea and its role has been described in the prevention and treatment of Alzheimer's disease [20]. It is also known as an aggregation inhibitor for both tau [21, 22] and amyloid-β [23, 24].

2. MATERIALS AND METHODS

2.1 Preparation of type 2 diabetes mellitus drugs for drug repurposing

DrugBank [25] was used to select small molecule-based approved drugs for type 2 diabetes mellitus. PubChem [26] was used to download the 3D SDF formats of 31 drugs to create a ligand library and identify the names and identifiers of all drug molecules. OpenBabel [27]: was used for all file format conversions. All drugs for type 2 diabetes mellitus in this study (1 Alogliptin, 2 Canagliflozin, 3 Chlorpropamide, 4 Dapagliflozin, 5 Empagliflozin, 6 Ertugliflozin, 7 Gemigliptin, 8 Gliclazide, 9 Glimepiride, 10 Glipizide, 11 Glyburide, **12 Linagliptin**, 13 Lipoic acid, 14 Luseogliflozin, 15 Metformin, 16 Miglitol, 17 Mitiglinide, 18 Nateglinide, 19 Pioglitazone, 20 Repaglinide, 21 Rosiglitazone, 22 Saxagliptin, 23 Simvastatin, 24 Sitagliptin, 25 Teneligliptin, 26 Tolazamide, 27 Tolbutamide, 28 Topiramate, 29 Trelagliptin, 30 Vildagliptin, 31 Voglibose) were selected according to the following rules: 1) The molecular weight of the drug must be less than 500 g/mol to cross the blood-brain barrier (BBB). 2) Drugs must belong to the small molecule class to allow more accurate prediction of binding modes. 3) All drugs must be approved for safe use. Whole drugs were prepared by adding polar hydrogens, merging nonpolar hydrogens, adding Gasteiger charges, and assigning the rotatable bonds of each molecule for docking with AutoDock and AutoDockTools. The prepared ligands were saved in PDBQT file format and docking procedures were performed for four aggregation-related segments of tau and amyloid-β. The same procedures were performed for the positive control ligand, EGCG. The top five ligands of the T2DM drugs in terms of binding free energy and their two-dimensional structures are shown in **Fig. 1**.

2.2 Preparation of tau and amyloid-β aggregation segments

The identified aggregation-prone peptides are from the Protein Data (RSCB PDB) [28] These peptides are related to both amyloid-β and tau proteins, which are the two most important proteins in aggregate formation. The four peptides include 37-GGVVIA-42 (PDB ID: 2ONV) and 16-KLVFFA-21 (PDB ID:3OVJ) from the amyloid-β protein; 275-VQIINK-280 (PDB ID: 5V5C) and 306-VQIVYK-311 (PDB ID: 3OVL) from the tau protein. The preparation steps of the peptides include removal of water molecules, addition of polar hydrogens and charges, and merging of nonpolar hydrogens. The peptides prepared for docking were stored in PDBQT file format for further steps.

2.3 Docking studies

Molecular docking studies were performed using AutoDock and AutoDockTools [29] on four targets with thirty-one T2DM drugs and one reference compound (EGCG). Before docking, the search space for the peptides was set to the entire target, including all residues for each peptide. Gridbox parameter files were saved in GPF file format and run through AutoGrid. The semi-flexible docking method (flexible ligand and rigid target) was used for the docking experiments. Docking parameter files were created in DPF file format according to this strategy and run through AutoDock. Ten unique conformations were generated using the default parameters of the docking tool and ordered by binding free energy. The AutoDock results file (DLG) was used to obtain information on hydrogen bonds, binding free energy values, conformations and ranking, and other calculated parameters. PyMOL was used to visualize the written complex structures to obtain relatively good resolution. Protein-Ligand Interaction Profiler (PLIP) was used to visualize and determine the secondary interactions of the complexes. PLIP only accepts the PDB files of the target ligand complex. For this purpose, the entire conformational files with the highest rank were written to PDBQT files and then converted to PDB files using OpenBabel. For the determination of non-covalent interactions in PLIP, the default parameters were used (maximum distance of hydrogen bonds is 4.1 Å, maximum distance of hydrophobic interaction is 4.0 Å, maximum distance of π-stacking is 5.5 Å). The whole results of the molecular interaction of the lead molecule linagliptin are shown in **Fig. 2** to **Fig. 5**.

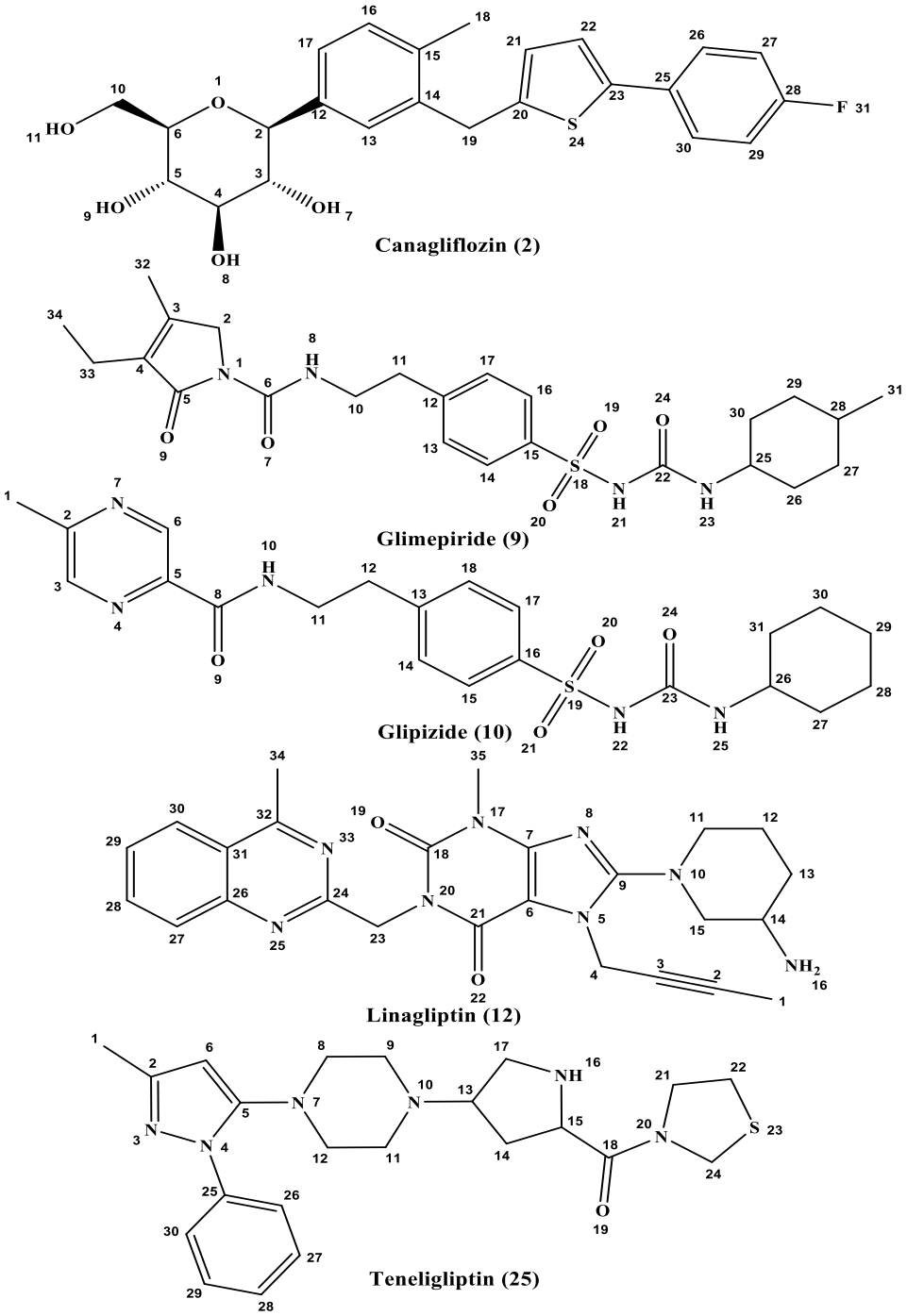


Fig. 1. The best ranked five type 2 diabetes drugs and their molecular structures with numbered style

3. RESULTS

In this study, the non-covalent interactions of five lead molecules with their targets were comprehensively examined. The main interaction forces were determined as hydrogen bonds, hydrophobic interactions, and π-stacking (π-π stacking). The highest mean value was determined for linagliptin, and the results were explained below.

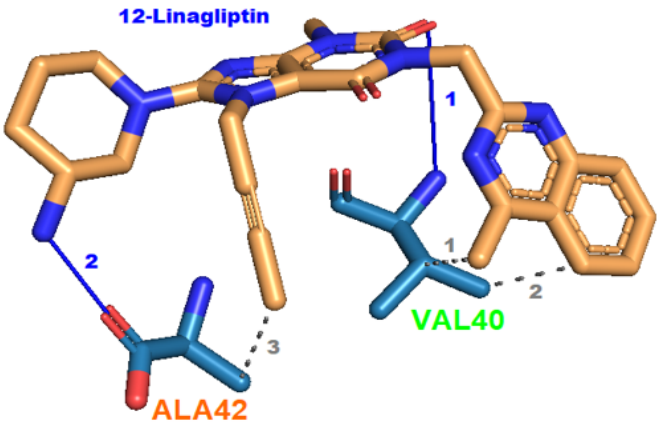
Linagliptin-GGVVIA complex: Five secondary interaction forces were determined, including three hydrophobic interactions (grey bonds in **Fig. 2**) and two hydrogen bonds (blue bonds in **Fig. 2**). The residues VAL40 and ALA42 are involved in bond formation with the O (Fig. 1, no 19) and NH (**Fig. 1**,

no 16) atoms of linagliptin. While oxygen (19) is involved as an acceptor in bond 1, NH (16) is involved as a hydrogen donor in bond 2. The hydrogen bond distances for 1 and 2 are calculated to be 3.17 and 1.90 Å, respectively.

Linagliptin-KLVFFA complex: Eight secondary interaction forces were determined, including four hydrophobic interactions, one hydrogen bond (**Fig. 3**), and three π-π stackings (green in Fig. 5). LEU17, PHE19, and ALA21 residues are involved in bond formation with the NH group of linagliptin (Fig. 1, no 16). There, NH (16) participated as a hydrogen donor. Hydrogen bond distance for 1 is calculated to be 1.77 Å. π-π Stacking interactions are formed between the quinazoline and pyrimidinedione rings of linagliptin and the aromatic ring of PHE19. According to the conformations formed between the aforementioned rings, π-π interactions 1 and 2 occurred face-to-face, whereas π-π interaction 3 occurred edge-to-face. The π-π interaction distances for 1, 2, and 3 are measured to be 4.13, 3.63, and 4.59 Å, respectively.

Linagliptin-VQIINK complex: Six secondary interaction forces were determined, including four hydrophobic interactions and two hydrogen bonds (**Fig. 4**). The residues GLN276, ILE278, and LYS280 are involved in the formation of bonds with the O (Fig. 1, no 19) and NH (Fig. 1, no 16) atoms of linagliptin. While oxygen (19) is involved as an acceptor in bond 1, NH (16) is involved as a hydrogen donor in bond 2. The hydrogen bond distances for 1 and 2 are measured to be 2.78 and 1.78 Å, respectively.

Linagliptin-VQIVYK complex: Two secondary interaction forces were determined, including one hydrophobic interaction and one hydrogen bond (**Fig. 5**). The residue VAL309 is included in the formation of bond with the N atom of linagliptin (Fig. 1, no 25). The nitrogen atom (25) participates in the interaction as a hydrogen bond acceptor. The hydrogen bond distance is measured to be 2.61 Å.



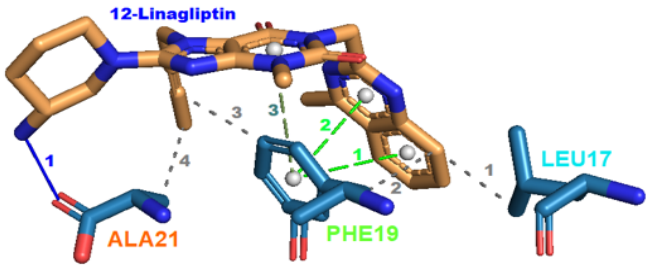
Hydrophobic interactions …

Index	Residue	AA	Distance	Ligand atom	Protein atom
1	40A	VAL	3.77	AlC	AlC
2	40A	VAL	3.22	ArC	AlC
3	42A	ALA	3.71	AlC	AlC

Hydrogen bonds —

Index	Residue	AA	Distance	Donor atom	Acceptor atom
1	40A	VAL	3.17	VAL NH	Lin O (19)
2	42A	ALA	1.90	Lin NH (16)	ALA O

Fig. 2. Interaction map and properties of 37-GGVVIA-42 (Aβ) and Linagliptin (12) complex



Hydrophobic interactions …

Index	Residue	AA	Distance	Ligand atom	Protein atom
1	17A	LEU	3.44	ArC	AlC
2	19A	PHE	3.97	ArC	AlC
3	19A	PHE	3.55	AlC	ArC
4	21A	ALA	3.65	AlH	AlC

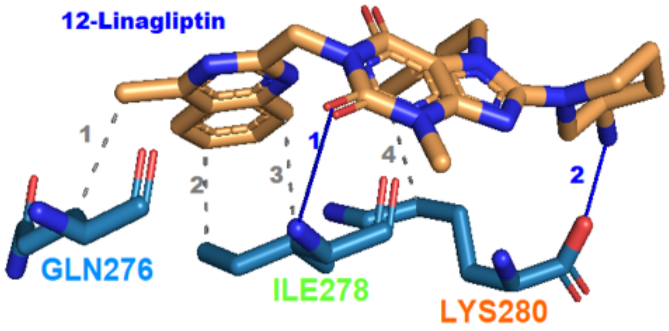
Hydrogen bonds —

Index	Residue	AA	Distance	Donor atom	Acceptor atom
1	21A	ALA	1.77	Lin NH (16)	ALA O

Π-Stacking …(parallel), …(perpendicular)

Index	Residue	AA	Distance	Ligand atom	Protein atom
1	19A	PHE	4.13	Lin [C26-C31]	PHE19
2	19A	PHE	3.63	Lin [C24-N33]	PHE19
3	19A	PHE	4.59	Lin [N17C18N20 C21C6C7]	PHE19

Fig. 3. Interaction map and properties of 16-KLVFFA-21 (Aβ) and Linagliptin (12) complex



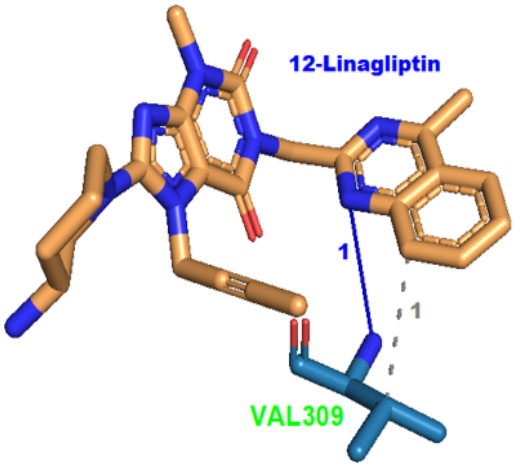
Hydrophobic interactions …

Index	Residue	AA	Distance	Ligand atom	Protein atom
1	276A	GLN	3.32	AlC	AlC
2	278A	ILE	3.22	ArC	AlC
3	278A	ILE	3.69	ArC	AlC
4	280A	LYS	3.70	AlC	AlC

Hydrogen bonds —

Index	Residue	AA	Distance	Donor atom	Acceptor atom
1	278A	ILE	2.78	ILE NH	Lin O (19)
2	280A	LYS	1.78	Lin NH (16)	LYS O

Fig. 4. Interaction map and properties of 275-VQIINK-280 (Tau) and Linagliptin (12) complex



Hydrophobic interactions ...

Index	Residue	AA	Distance	Ligand atom	Protein atom
1	309A	VAL	3.71	AlC	ArC

Hydrogen bonds —

Index	Residue	AA	Distance	Donor atom	Acceptor atom
1	309A	VAL	2.61	VAL NH	Lin N (25)

Fig. 5. Interaction map and properties of 306-VQIVYK-311 (Tau) and Linagliptin (12) complex

The results below were obtained from molecular docking studies performed on four fibrils, which are shown pioneers for aggregation forming, with thirty-one T2DM drugs and one positive reference compound. For five lead drugs, docking results are given in **Table 1**, including various docking parameters. EGCG, which is reported as an aggregation inhibitor, and thirty-one T2DM drugs were docked on four unique fibril targets, and the results were compared with a positive control compound. According to the getting results, for each target were found at least four T2DM drugs with higher binding activity than EGCG. Twenty-eight drugs for GGVVIA, twenty-nine drugs for KLVFFA, four drugs for VQIINK, and twenty-four drugs for VQIVYK have binding affinity a higher than EGCG. Scoring functions of complexes changed from -1.70 kcal/mol (voglibose, 31) to -5.37 (linagliptin, 12) for 37-GGVVIA-42, from -2.22 (voglibose, 31) to -5.68 (canagliflozin, 2) for 16-KLVFFA-21, from -1.82 (miglitol, 16) to -5.39 (linagliptin, 12) for 275-VQIINK-280, from -1.91 (miglitol, 16) to -5.72 kcal/mol (teneligliptin, 25) for 306-VQIVYK-311. For each target, the top nine results were listed in **Table 2**. With these results, it was determined common eight drugs (1, 2, 9, 10, 12, 25, 29, 30) for amyloid-β fibrils (GGVVIA and KLVFFA), common six drugs (2, 8, 9, 10, 12, 25) for tau fibrils (VQIINK and VQIVYK), and common five (2, 9, 10, 12, 25) drugs for all targets (GGVVIA, KLVFFA, VQIINK, and VQIVYK). Mean values of the determined five common drugs were calculated for four targets and given in **Table 2**. According to these values, linagliptin ranked first with -5.337 kcal/mol, glimepiride ranked second with -5.177 kcal/mol, teneligliptin ranked third with -5.135 kcal/mol, canagliflozin ranked with -5.085 kcal/mol, and glipizide ranked last with -4.990 kcal/mol. The compounds also have lower mean free binding energy values than the mean value of EGCG (**Table 2**).

Drugs	Canagliflozin (2)	Glimepiride (9)	Glipizide (10)	Linagliptin (12)	Teneligliptin (25)	EGCG (positive control)
Target (hexapeptide)			**Aβ (37-GGVVIA-42)**			
Binding energy	-4.41	-4.9	-4.22	-5.37*	-4.86	-2.92
Ligand efficiency	-0.14	-0.14	-0.14	-0.15	-0.16	-0.09
Inhibitor constant (μM)	582.82	257.29	811.34	115.13	275.52	7.27
Intermolecular energy	-6.8	-6.99	-6.13	-7.16	-6.05	-6.5
Vdw-hb desolvation energy	-6.32	-6.99	-6.13	-5.34	-4.63	-6.19
Electrostatic energy	-0.48	0.01	0.17	1.82	1.42	0.31
Total internal energy	-2.63	-1.9	-1.77	-1.33	-2.58	-5.25
Torsional energy	2.39	2.09	2.09	1.79	1.19	3.58
Unbound energy	-2.63	-1.9	-1.77	-1.33	-2.58	-5.25
CIRMS	0.0	0.0	0.0	0.0	0.0	0.0
refRMS	12.53	9.78	8.62	11.8	12.95	10.79
Target (hexapeptide)			**Aβ (16-KLVFFA-21)**			
Binding energy	-5.68*	-5.32	-5.46	-5.19	-5.32	-3.29
Ligand efficiency	-0.18	-0.16	-0.18	-0.15	-0.18	-0.1
Inhibitor constant (μM)	68.4	126.66	98.68	157.62	125.82	3.85
Intermolecular energy	-8.07	-7.41	-7.55	-6.98	-6.51	-6.87
Vdw-hb desolvation energy	-7.81	-7.34	-7.39	-5.12	-5.25	-6.58
Electrostatic energy	-0.26	-0.06	-0.16	-1.85	-1.26	-0.29
Total internal energy	-3.26	-1.01	-1.75	-1.17	-2.28	-4.83
Torsional energy	2.39	2.09	2.09	1.79	1.19	3.58
Unbound energy	-3.26	-1.01	-1.75	-1.17	-2.28	-4.83
CIRMS	0.0	0.0	0.0	0.0	0.0	0.0
refRMS	9.53	12.14	13.19	8.52	7.41	7.97
Target (hexapeptide)			**Tau (275-VQIINK-280)**			
Binding energy	-5.23	-5.25	-4.73	-5.39*	-4.64	-5.00
Ligand efficiency	-0.17	-0.15	-0.15	-0.15	-0.15	-0.15
Inhibitor constant (μM)	147.54	142.69	342.22	111.77	398.76	216.67
Intermolecular energy	-7.61	-7.33	-6.82	-7.18	-5.83	-8.58
Vdw-hb desolvation energy	-7.52	-7.36	-6.80	-5.49	-5.78	-8.04
Electrostatic energy	-0.1	-0.03	-0.02	-1.69	-0.05	-0.54
Total internal energy	-2.66	-2.37	-2.03	-1.24	-2.13	-4.94
Torsional energy	2.39	2.09	2.09	1.79	1.19	3.58
Unbound energy	-2.66	-2.37	-2.03	-1.24	-2.13	-4.94
CIRMS	0.0	0.0	0.0	0.0	0.0	0.0
refRMS	13.54	14.60	14.22	15.36	12.90	12.51
Target (hexapeptide)			**Tau (306-VQIVYK-311)**			
Binding energy	-5.02	-5.24	-5.55	-5.40	-5.72*	-3.90

Ligand efficiency	-0.16	-0.15	-0.18	-0.15	-0.19	-0.12
Inhibitor constant (μM)	207.5	144.36	85.36	109.27	64.25	1.39
Intermolecular energy	-7.41	-7.33	-7.64	-7.19	-6.91	-7.48
Vdw-hb desolvation energy	-7.27	-7.09	-7.58	-5.74	-5.91	-7.28
Electrostatic energy	-0.14	-0.23	-0.05	-1.45	-1.00	-0.20
Total internal energy	-2.48	-1.86	-1.53	-1.2	-1.70	-4.87
Torsional energy	2.39	2.09	2.09	1.79	1.19	3.58
Unbound energy	-2.48	-1.86	-1.53	-1.20	-1.70	-4.87
CIRMS	0.0	0.0	0.0	0.0	0.0	0.0
refRMS	12.52	11.33	10.34	13.87	14.66	9.45

* indicates the highest binding energy score in 31 type-2 diabetes mellitus drugs

Table 1. Docking results of five lead T2DM drugs and EGCG on aggregation-forming segments

	Amyloid-β				**Tau**			
	37-GGVVIA-42		**16-KLVFFA-21**		**275-VQIINK-280**		**306-VQIVYK-311**	
	Compound	ΔG value	Compound	ΔG value	Compound	ΔG value	Compound	ΔG value
Positive control	EGCG	-2.92	EGCG	-3.29	EGCG	-5.00	EGCG	-3.90
Drugs (first common 9)	12	-5.37	2	-5.68	12	-5.39	25	-5.72
	9	-4.90	10	-5.46	9	-5.25	10	-5.55
	25	-4.86	9, 25	-5.32	2	-5.23	12	-5.40
	30	-4.54	23	-5.24	8	-5.04	9	-5.24
	29	-4.43	12	-5.19	10	-4.73	8	-5.19
	2	-4.41	30	-5.18	4	-4.71	26	-5.07
	1	-4.34	7	-5.14	21	-4.69	2	-5.02
	8	-4.27	24	-4.89	25 and 30	-4.64	1	-4.94
	10 and 22	-4.22	1 and 29	-4.81	26	-4.63	17	-4.87
Common Drugs	1, 2, 9, 10, 12, 25, 29, 30 (*)				2, 8, 9, 10, 12, 25 (**)			
Common Drugs	2, 9, 10, 12, 25 (***)							
Common for *GGVVIA/KLVFFA, **VQIINK/VQIVYK, *** GGVVIA, KLVFFA, VQIINK, and VQIVYK								
	Mean values for first five lead molecules							
Drug number	**12**	9	25	2	10	EGCG		
Mean ΔG	**-5.337**	-5.177	-5.135	-5.085	-4.990	3.777		
Name	**linagliptin**	glimepiride	teneligliptin	canagliflozin	glipizide	Epigallocatechin Gallate		

Table 2. Docking evaluations for top nine T2DM drugs and determination of multitarget drug candidates for AD

4. DISCUSSION

By specifically inhibiting all possible aggregation-forming targets, we may have a more effective way to combat neurofibrillary tangles and amyloid fibrils in Alzheimer's disease. With this in mind, we investigated whether we could find common inhibitor candidates for aggregation formation in 31 T2DM drugs. We can advise wet lab scientists to evaluate these five drugs (linagliptin, glimepiride, teneligliptin, canagliflozin, and glipizide) for further experimental studies in Alzheimer's disease. There are other experimental studies on the use of T2DM drugs in Alzheimer's disease [30-32]. These studies are consistent with our findings for the above drugs. For example, the dipeptidyl peptidase-4 inhibitor linagliptin has shown beneficial effects on cognitive performance [33-35]. Glimepiride reduces amyloid-β production by suppressing BACE1 [36], shows AChE inhibitory effects [37], and protects neurons from synapse damage [38]. According to a computational study, canagliflozin could be an AChE inhibitor [39]. According to our and other results, antidiabetic drugs show similar effects on many AD-related targets. For this reason, multitarget approaches to T2DM drugs are a reasonable choice for further study. We searched the five drugs for the treatment of Alzheimer's disease from ClinicalTrials.gov (access date: 04.03.2020) but could not find any clinical records of these drugs for the treatment of Alzheimer's disease.

5. CONCLUSION

In the presented study, in silico drug repurposing strategy has been designed on T2DM drugs included small molecules, to use them against Alzheimer's disease. In the target choice, aggregation inhibition treatment strategy has been adopted, and aggregation pioneer segments from tau and amyloid-β proteins have been determined and chosen from RSCB PDB. The related segments are 37-GGVIA-42 (PDB code: 2ONV) and 16-KLVFFA-21 (PDB code: 3OVJ) in amyloid-β protein, and 275-VQIINK-280 (PDB code: 5V5C) and 306-VQIVYK-311 (PDB code: 3OVL) in tau protein. Molecular docking experiments have been performed by the AutoDock 4.2 docking tool. According to the docking results, five lead T2DM drugs have been determined compared to the positive control compound, EGCG. Linagliptin (compound no: 12) has been determined as an MTDL ligand with the lowest mean binding free energy value on four targets. The remaining four drugs are glimepiride, teneligliptin, canagliflozin, and glipizide.

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