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RESEARCH ARTICLE

In silico Pharmacokinetics and Molecular Docking of Selected Compounds against Target Proteins of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial disease, which can be simply stated as a progressive and irreversible chronic disease of aging. The main purpose of the existing work was to find out the anti-Alzheimer's activity of repurposing drugs and novel chemical compounds. In the present *in silico* study, IQ3, echothiophate, 15d-PGJ2, mefenamic acid, phentolamine, and nateglinide were screened on four major protein targets AChE, beta-secretase 1, C-Jun N-terminal kinase-3, and peroxisome proliferator-activated receptor γ by molecular docking using Autodock Vina software. The ligands are then compared to the well-known standard inhibitors of their specific proteins. Using pkCSM and SwissADME software, pharmacokinetic properties were also analyzed. In accordance with the molecular docking scores, out of the screened ligands IQ3, nateglinide, phentolamine, and mefenamic acid significantly linked with chosen targets of AD. In the present study, no drug violates Lipinski's fifth rule. All the ligands have blood–brain barrier permeability and intestinal absorption. Toxicity prediction results showed that all ligands are non-hepatotoxic with the exception of ligand IQ3, and no AMES toxicity was observed with the exception of IQ3, phentolamine. The current study suggested that among the six ligands evaluated, nateglinide and mefenamic acid may be effective in improving memory in AD and dementia on the basis of molecular docking and pharmacokinetic parameters.

Keywords: Alzheimer's disease, Repurposing drugs, Acetylcholinesterase, β-Secretase 1, C-Jun N-terminal kinase-3, Peroxisome proliferator activated receptor γ, SwissADME, AutoDock Vina

INTRODUCTION

Alzheimer's disease (AD) is a progressive, irreversible chronic disease of aging described by increasing cognitive impairment, aphasia (unable to understand or produce speech, as a result of brain damage), agnosia (impaired ability to process sensory information), and difficulties with the daily living activities.[1] Worldwide, nearly 46 million people are living with dementia and there are around 10 million new cases annually.[1,2] The world Alzheimer's report 2015 was an investigation of the incidence, prevalence, cost, and trends in AD. The report estimated that by the year 2050, this figure would increase to above 131.5 million.[3] In the WHO's 2019 health estimates released recently, based on that report, dementia is also one of the world's top 10 causes of death. AD name was coined by scientist named Alois Alzheimer in 1907.[4]

AD may be caused due to extracellular deposition of beta-amyloid and intracellular accumulation of neurofibrillary tangles with hyperphosphorylated tau protein, cholinergic dysfunction, inflammation, and oxidative stress.[5] AD can be late-onset, sporadic or early-onset, and familial type.[6] In

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AD, difficulty in remembering the recent events is most commonly seen early symptom. In most cases, people having disease those with the late-onset type at their mid-60s symptoms first appear. Early-onset (young onset) AD occurs at the age of person's between 30s and mid-60s and this type is very rare.[7] While its incidence is largely increasing, there is a great need to develop new anti-Alzheimer's drugs.[8] JNK 3 inhibitors (JNK3Is), acetylcholinesterase inhibitors, beta-secretase 1 inhibitors (BACE1), and peroxisome proliferator-activated receptor gamma agonists (PPAR-γ) are also considered as a major targets to treat AD.

Acetylcholinesterase is an enzyme belongs to the family of serine hydrolase that breaks the neurotransmitter acetylcholine into acetate and choline.[9,10] Several studies suggested that decline of acetylcholine causes memory impairment. Therefore, AChE inhibition should be considered to successfully improve acetylcholine (Ach) levels in the synaptic cleft.[11] Amyloid beta (Aβ) is generated by the endoproteolysis of Aβ precursor protein (APP). β-Secretase 1 (BACE1) is an aspartic acid protease involved in the breakdown of the transmembrane APP.[12] C-Jun N-terminal kinases (JNKs) belongs to the mitogen-activated protein kinases (MAPKs). JNKs (or stress-activated protein kinase) have three forms JNK1, JNK2, and JNK3.[13] These are activated by environmental stress, UV radiation, growth factors, and cytokines. Particularly JNK3 (found in brain, heart, and testes) involved in phosphorylation of tau proteins and enhancing Aβ production, it can also raise BACE-1 expression.[14-16] Nuclear receptors such as PPAR-γ is reported to play an important role in the lipid, glucose, and energy metabolism in the brain, it reduces the synthesis of Aβ, regulates mitochondrial biogenesis, and prevents neuroinflammation, contributing to improved cognitive function in AD.[17-19]

The present study is designed to evaluate the anti-Alzheimer effect of selected ligands some are repurposing drugs such as mefenamic acid (NSAIDs), phentolamine (antihypertensive), nateglinide (antidiabetic), and echothiophate (glaucoma), other ligands are novel molecules IQ3, 15d-PGJ2 as a multitarget inhibitors,[20] the bioactivity of ligands was studied *in silico* using several screening methods, which include molecular docking, druglikeness, and toxicological screening. Drug repurposing is the process of evaluating the effectiveness of a drug that is already known for its new therapeutic role.[21] These ligands are selected because IQ3 is a JNK3Is, JNK 3 was associated with beta-amyloid in senile plaques.[12] Echothiophate is acetylcholinesterase inhibitor, acetylcholine levels are decreased in AD patients.[22] 15d-PGJ2 is a PPAR-γ agonist, it reduces the beta-amyloid plaques.[13] Mefenamic acid is a COX inhibitor, inflammation is the pathological hallmark of AD.[23] Phentolamine is a α2 adrenergic blocker, α2 adrenergic receptor activation enhances amyloidogenic processing of amyloid precursor protein (APP), results Aβ load in the brain.[24] Nateglinide is a protein-tyrosine phosphatase 1B (PTP1B) inhibitor, neuroinflammation and stress of the endoplasmic reticulum both are associated with amyloidosis mainly observed in AD, results in increased activity of the PTP1B. It further activates the pro-inflammatory response of microglia.[25]

METHODOLOGY

Equipment

Molecular docking studies were performed using a laptop with CORE i-7 processor specifications, 4 GB RAM, and Windows 10 OS. The software used includes Discovery Studio Visualizer v20.1.0.19295, MGLTools 1.5.6 (The Scripps Research Institute), package which consists of Autodock (http://mgltools.scripps.edu/downloads), Autodock Vina (http://vina.scripps.edu/), Pymol (www.pymol.org), Protein Data Bank (https://www.rcsb.org/), SwissADME (http://www.swissadme.ch/),pkCSM(http://biosig.unimelb.edu.au/pkcsm/prediction), and PubChem (http://PubChem. ncbi.nlm. nih. gov).

Protein preparation

The X-ray crystallographic structure of four protein molecules acetylcholinesterase at resolution 2.00

Å (PDB ID 4M0E), β-Secretase at resolution 1.90 Å (1FKN), MAPKs 10 at resolution 2.00 Å (PDB ID 4KKH), and PPAR gamma at resolution 2.00 Å (5YCP) as targets were downloaded from protein data bank (PDB)(http://www.rcsb.org/) in.pdb format. Before performing molecular docking, all the protein structures were purified, water molecules were removed, Kollman charges and polar hydrogens were added, and non-polar hydrogens merged using AutoDock Vina. The targets were selected depending on the source organism, resolution. The details of which are given in Table 1.

Ligand (guest) preparation

Six ligands IQ3, echothiophate, mefenamic acid, phentolamine, 15d-PGJ2, and nateglinide were selected in the present study. The 3D crystal structure of ligand molecules was obtained from PubChem (www.pubchem.ncbi.nlm.nih.gov/). CI files have been converted to three-dimensional (3D) structures using pymol software (www.pymol.org) and saved as.*pdb* format, and prepared for docking studies by adding Gastier charges, merged non-polar hydrogens, and saved as.*pdbqt* format. Details of the ligands are given in Table 2.

Docking studies

It is a powerful tool in testing ligand binding to the active site of an enzyme or receptor. The molecular docking was performed using Autodock Vina software to find out the docking values. The best docking values, in terms of binding free energy (expressed as more negative values), were assessed for further analysis.[30]

ADME/T property prediction

In silico ADME/T studies are designed to accurately evaluate *in vivo* pharmacokinetic properties and toxicity of drug molecules.[31] pkCSM online database (http://biosig.unimelb.edu.au/pkcsm/prediction) has been used to investigate the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of ligand molecules.[32]

Druglikeness

Using Swiss ADME software ligands were evaluated for druglikeness using Lipinski (Pfizer), Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer) criteria which was predicted. Druglikeness of the ligands is observed in Table 3.[15]

Table 1: Details of the receptors used in the study

S. No.	Receptor (PDB ID)	Cocrystalized ligand	Resolution; R-free; R-factor	References
1.	Acetylcholinesterase (4M0E)	Dihydrotanshinone I and Territrem B	2.00 Å; 0.160; 0.196	Cheung *et al*.[26]
2.	β-Secretase (1FKN)	Memapsin	1.90 Å; 0.224; 0.180	Hong *et al*.[27]
3.	JNK-3 (4KKH)	AMP, apo	2.00 Å; 0.277; 0.229	Han *et al*.[28]
4.	PPAR gamma (5YCP)	Rosiglitazone	2.00 Å; 0.233; 0.201	Jang *et al*.[29]

Table 2: Details of the ligands in the current study

S. No.	Ligand	PubChem (ID)	Mol. Wt. (g/mol)	Molecular formula	HBD	HBA	RB	Type of activity
1.	Donepezil	CID_3152	379.5	$C_{24}H_{29}NO_3$	0	4	6	AChE inhibitor
2.	Semagacestat	CID_9843750	361.4	$C_{19}H_{27}N_3O_4$	3	4	5	β-Secretase inhibitor
3.	SP600125	CID_8515	220.23	$C_{14}H_8N_2O$	1	2	0	JNK 3 inhibitor
4.	Rosiglitazone	CID_77999	357.4	$C_{18}H_{19}N_3O_3S$	1	6	7	PPAR-γ agonist
5.	Echothiophate	CID_10548	256.33	$C_9H_{23}NO_3PS^+$	0	4	8	AChE inhibitor
7.	Nateglinide	CID_5311309	317.4	$C_{19}H_{27}NO_3$	2	3	6	PTP1B inhibitor
8.	IQ 3	CID_777728	341.3	$C_{20}H_{11}N_3O_3$	0	6	3	JNK 3 inhibitor
9.	15d-PGJ2	CID_5311211	316.4	$C_{20}H_{28}O_3$	1	3	11	PPAR-γ agonist
10.	Phentolamine	CID_5775	281.35	$C_{17}H_{19}N_3O$	2	3	4	α2 adrenergic blocker
11.	Mefenamic acid	CID_4044	241.28	$C_{15}H_{15}NO_2$	2	3	3	COX inhibitor

HBD: Hydrogen bond donor, HBA: Hydrogen bond acceptor, RB: Rotatable bonds

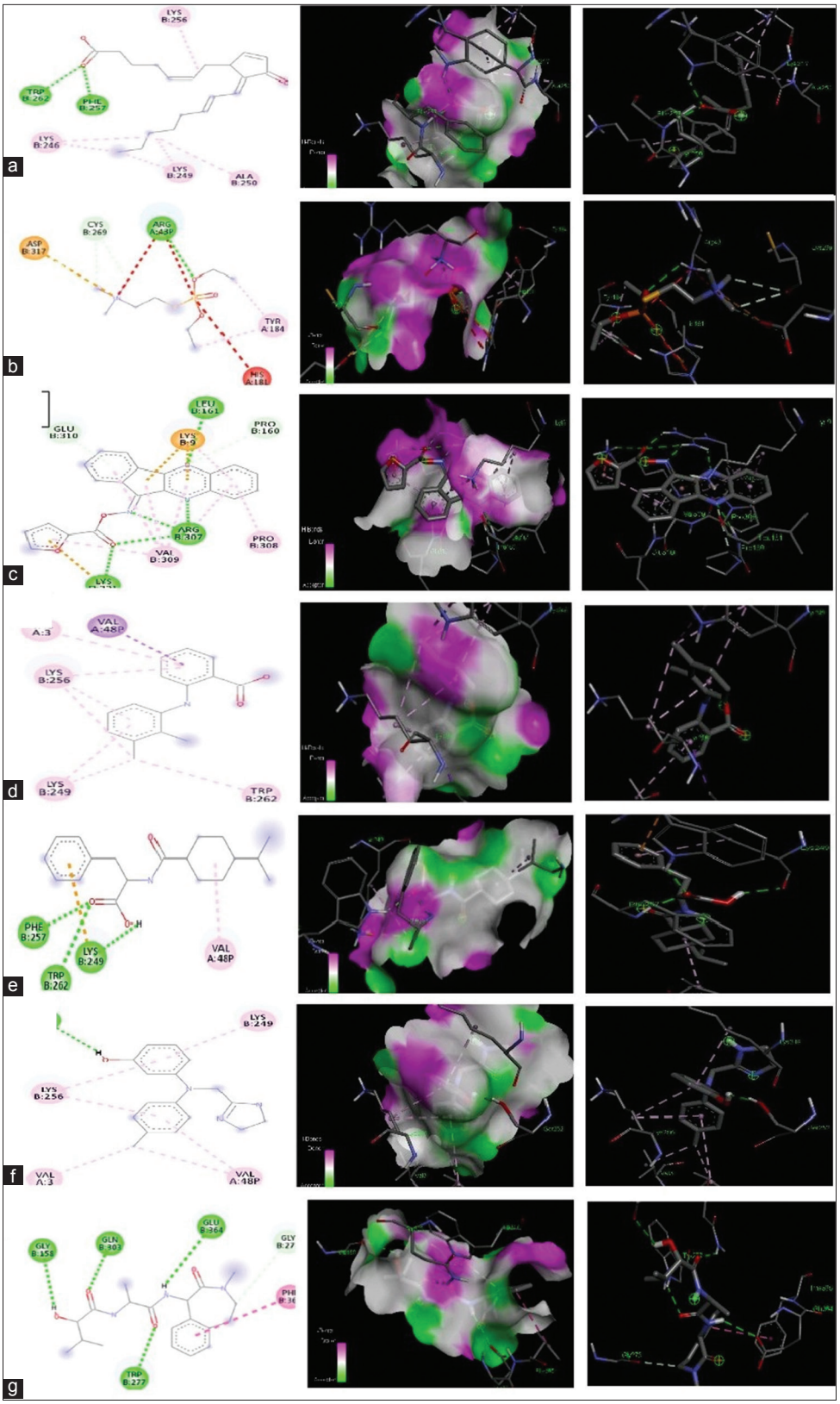

Figure 1: The two-dimensional and three-dimensional views of (a) 15d-PGJ2, (b) echothiophate, (c) IQ3, (d) mefenamic acid, (e) nateglinide, (f) phentolamine, and (g) rosiglitazone interactions with β-Secretase (1FKN) using AutoDock Vina software

Table 3: Druglikeness violation of ligands

Compound	Number of violations					Bioavailability
	LIPINSKI	GHOSE	VEBER	EGAN	MUEGGE	
IQ3	-	-	-	-	-	0.55
Echothiophate	-	-	-	-	-	0.55
15d-PGJ2	-	-	-	-	-	0.55
Mefenamic acid	-	-	-	-	-	0.55
Phentolamine	-	-	-	-	-	0.55
Nateglinide	-	-	-	-	-	0.55

RESULTS AND DISCUSSION

The main focus of the current study is to identify ligands (some are novel and some are repurposing) for the treatment of AD. Six ligands were tested by molecular docking. Out of these drugs, IQ3, nateglinide, phentolamine, and mefenamic acid have been significantly interacted with the selective targets of AD proteins.

Molecular docking study

The best pose docks core compounds were predicted for the interaction with AD targets of AChE (PDBID: 4M0E), β-Secretase (PDBID:1FKN), and JNK- 3 (PDBID:4KKH).

Figure 1 ligands were screened against the four selected targets of the A Drecep and the results of both docking scores and amino acid inter actions are shown in Table 4. In case of AChE (4M0E), −5.3–−9.0 in case of β-Secretase (1FKN), −4.1–−8.8 in JNK-3 (4KKH), −4.7–−9.6 in PPAR gamma (5YCP), and −4.3–8.5. Among these ligands, the best it for each target was selected on the basis of docking score and binding energy. The order of the ligands showed docking score against the AD targets was IQ3>Nateglinide>Phentolamine>Mefenamic acid>15d-PGJ2>Echothiophate. The ligands were also compared with potent known drugs/inhibitors of the target protein.

Dock score interaction with 4 M0E

In case of acetyl-coA lines terase, the molecular docking score of Q3 is –9.0, echothiophate is -5.0,15d-PGJ2 is -6.3, mefenamic acid is -7.0, and phentolamine is -6.9, in case of nateglinide, it showed –8.0. IQ3 showed the highest docking score when compared to standard drug donepezil (–8.6).

Dock score interaction with 1FKN

In case of β-Secretase, the molecular dockings core of IQ3 is -8.8, echothiophate is –4.1, 15d-PGJ2 is –5.5, mefenamic acid is –6.0, and phentolamine is –6.5, in case of nateglinide, it shows -6.8. IQ3 shows the highest docking score when compared to standard drug semagacestat (–6.9).

Dock score interaction with 4KKH

In case of JNK3, the molecular docking score of IQ3 is -9.6, echothiophate is –4.7, 15d-PGJ2 is –6.5, mefenamic acid is –7.1, and phentolamine is –7.5, in case of nateglinide, it shows –7.7. IQ3 and nateglinide show the highest docking score when compared to standard drug SP600125 (–7.6).

Dock score interaction with 5YCP

In case of PPAR-γ, the molecular docking score of IQ3 is –8.5, echothiophate is –4.3,15d-PGJ2 is –6.3, mefenamic acid is –6.6, and phentolamine is –6.6, in case of nateglinide, it shows –7.5. IQ3 and nateglinide show the highest docking score and phentolamine and mefenamic acid show equal dock score when compared to standard drug rosiglitazone (–6.6).

Predicting pharmacokinetic properties

Pharmacokinetics depends on the number of molecular descriptors of the target drug. *In silico* prediction of ADME/PK parameters has become

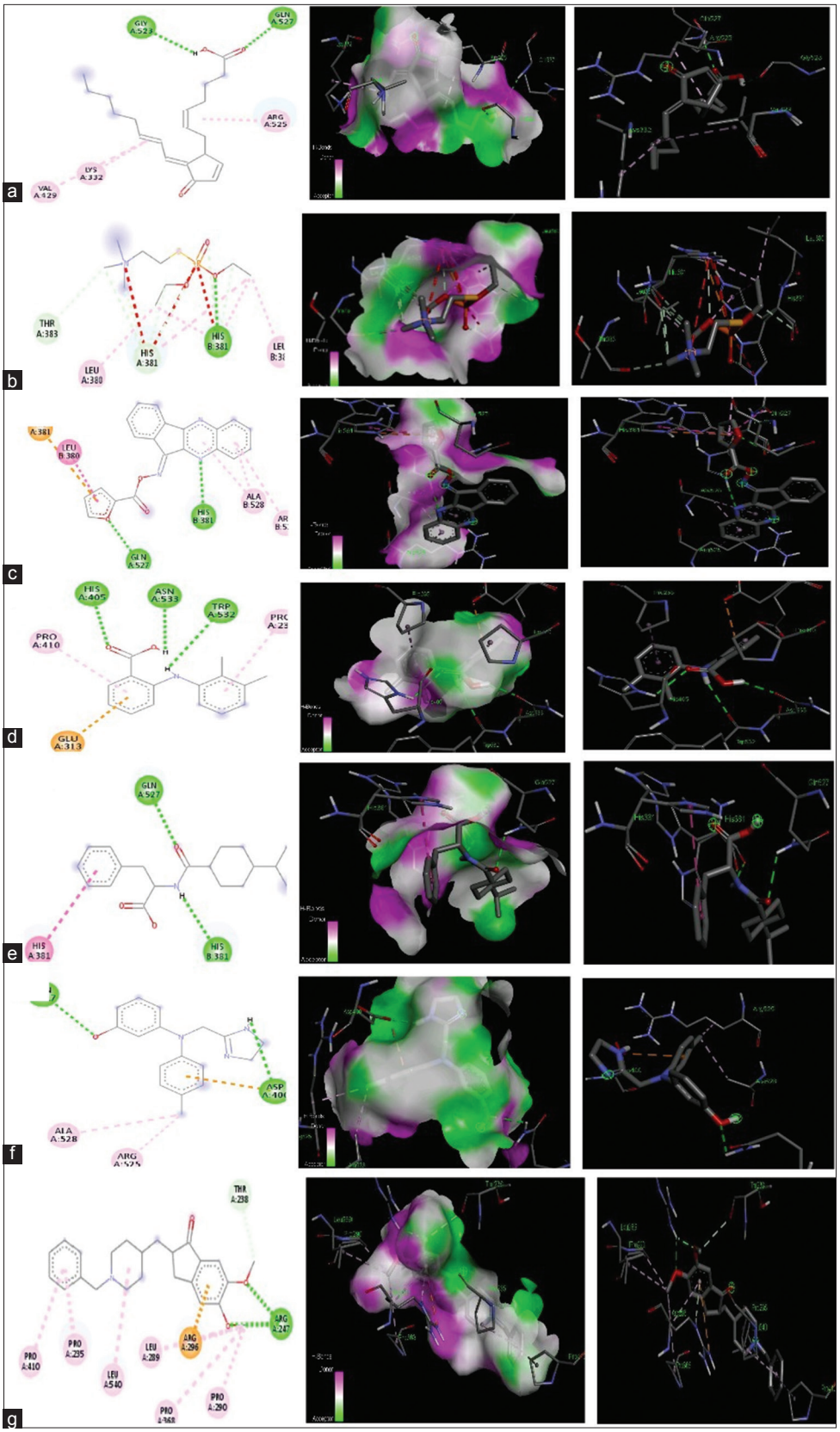

Figure 2: The two-dimensional and three-dimensional views of (a) 15d-PGJ2, (b) echothiophate, (c) IQ3, (d) mefenamic acid, (e) nateglinide, (f) phentolamine, (g) rosiglitazone interactions with acetylcholinesterase (4M0E) using AutoDock Vina software

increasingly important in drug selection and determining its effectiveness in human therapeutic use. Therefore, these physiochemical properties were calculated to determine ADME drug

Table 4: Docking scores and amino acid interactions of ligands

S.No.	PDB ID	Ligand	Affinity (kcal/mol)	Hydrogen bond	Residual hydrophobic/Pi-Cation/Pi-Anion/Pi-Alkyl interactions/Pi-Pi stacked interactions
1.	4M0E	IQ3	–9.0	HIS B:381, GLN A:527	HIS A:381, LEU B:380, ALA B:528, ARG B:525
		Echothiophate	–5.3	HIS B:381	HIS A:381, LEU B:380, LEU A:380, THR A:383
		15d-PGJ2	–6.3	GLY A:523, GLN A:527	ARG A:525, LYS A:332, VAL A:429
		Mefenamic acid	–7.0	HIS A:405, ASN A:533, TRP A:532	PRO A:235, PRO A:410, GLU A:313
		Phentolamine	–6.9	ASP A:400, GLN B:527	ALA A:528, ARG A:525
		Nateglinide	–8.0	HIS B:381, GLN A:527	HIS A:381
		Donepezil	–8.6	ARG A:247	THR A:238, PRO A:290, ARG A:296, PRO A:368, LEU A:289, LEU A:540, PRO A:235, PRO A:410
2.	1FKN	IQ3	–8.8	LEU B:161, ARG B:307, LYS B:321	PRO B:160, GLU B:310, LYS B:9, VAL B:309, PRO B:308
		Echothiophate	–4.1	ARG A:43P	CYS B:269, ASP B:317, TYR A:184,
		15d-PGJ2	–5.5	PHE B:257, TRP B:262	LYS B:256, ALA B:250, LYS B:246, LYS B:249
		Mefenamic acid	–6.0	-	VAL A:48P, VAL A:3, LYS B:256, LYS B:249, TRP B:262
		Phentolamine	–6.5	SER B:252	LYS B:249, LYS B:256, VAL A:3, VAL A:48P
		Nateglinide	–6.8	LYS B:249, TRP B:262, PHE B:257	VAL A:48P
		Semagacestat	–6.9	GLY B:158, GLN B:303, GLU B:364, TRP B:277	GLY B:273, PHE B:365
3.	4KKH	IQ3	–9.6	ARG A:107	ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230
		Echothiophate	–4.7	LYS A:93, ARG A:107, ASN A:194	LEU A:206, ASP A:207, GLU A:111
		15d-PGJ2	–6.5	LYS A:93, LYS A:191, ASN A:194	ALA A:74, LEU A:95, TYR A:223
		Mefenamic acid	–7.1	LYS A:93	SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74
		Phentolamine	–7.5	ARG A:107	LYS A:106, TYR A:103, GLY A:209, LEU A:210, ALA A:214, ALA A:211
		Nateglinide	–7.7	-	LYS A:93, ALA A:74, TYR A:223
		SP600125	–7.6	ARG A:107	LEU A:206, LYS A:93, ALA A:74
4.	5YCP	IQ3	–8.5	SER A:342	ARG A:288, LYS A:261, ILE A:341, BRL A:301
		Echothiophate	–4.3	-	ARG A:280, PHE A:247, GLU A;259, LYS A:261
		15d-PGJ2	–6.3	-	LYS A:261, ILE A:341, BRL A:501
		Mefenamic acid	–6.6	SER A:342, GLY A:258, LYS A:261	GLU A:259
		Phentolamine	–6.6	-	ARG A:288, PHE A:287, BRL A:501
		Nateglinide	–7.5	GLU A:259	ILE A:341, ARG A:280
		Rosiglitazone	–6.6	SER A:342	GLU A:259, BRL A:501

properties.[21] The ligands were studied for their ADME/T prediction; the data are shown in Table 5. Lipinski's RO5 helps to evaluate the druglikeness and it was based on the recognition that drug with a molecule mass; less than 500 Daltons, hydrogen bond donors; no more than 5, hydrogen bond acceptors; no more than 10 and octanol-water partition coefficient (log p); not exceed 5 can be administered orally. Molecules that do not follow more than 1 of these four rules may have a problem with bioavailability.[33] In the present work, none of the drugs violate the Lipinski's rule of five. All the ligands have BBB permeability and intestinal absorption. In metabolism, no ligand was a substrate for CYP2D6. IQ3, 15d-PGJ2, and phentolamine exhibited CYP3A4 substrate positive. IQ3 predicted as a 1A2, 2C9, and 2C19 inhibitor. Phentolamine was 1A2, 2C19, and 2D6 inhibitor and mefenamic acid predicted as a 1A2 and 2C9 inhibitor. The excretion property showed that none of the ligands were substrate for renal organic cation transporter 2 (OCT2) except

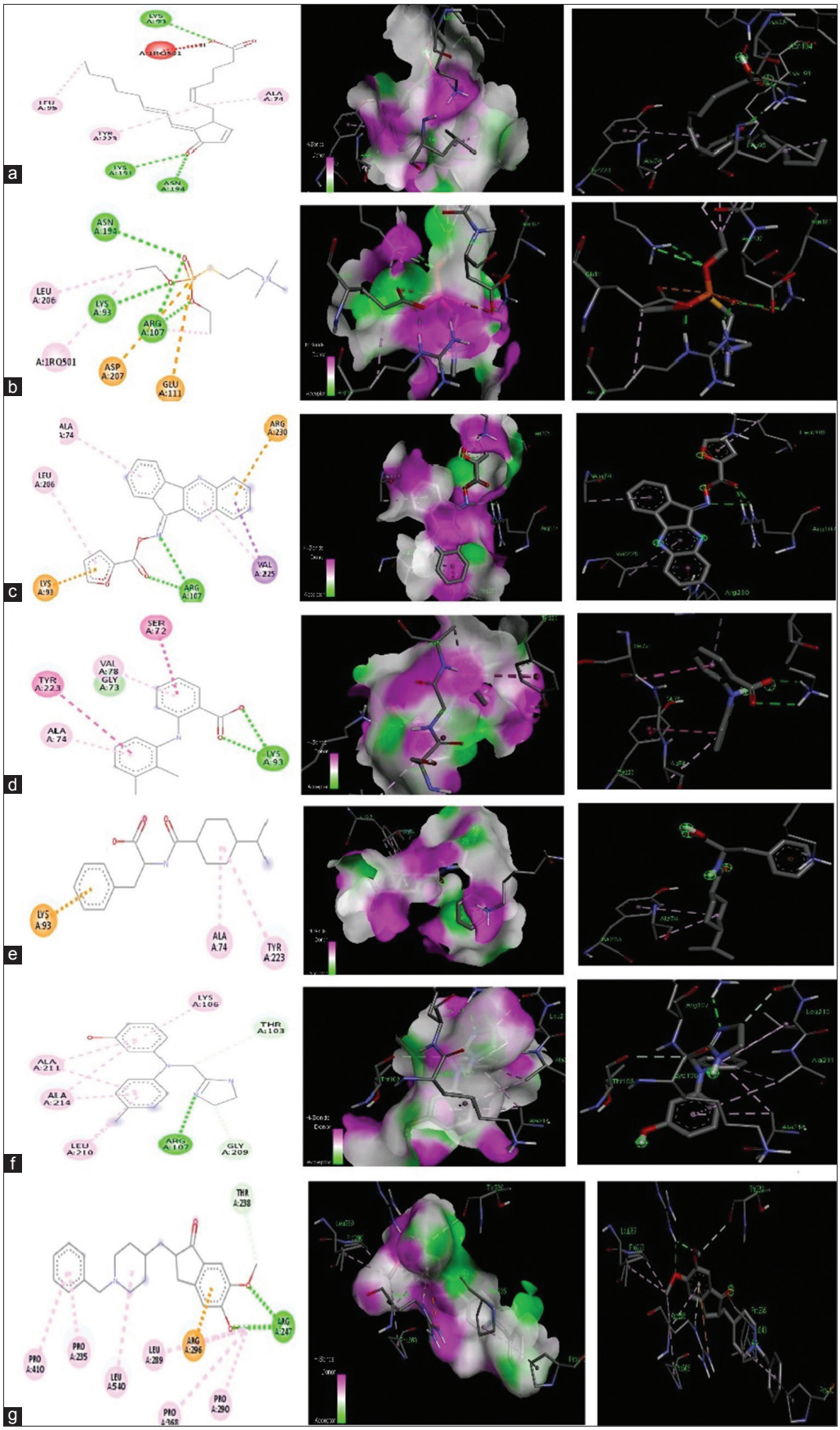

Figure 3: The two-dimensional and three-dimensional views of (a) 15d-PGJ2, (b) echothiophate, (c) IQ3, (d) mefenamic acid, (e) nateglinide, (f) phentolamine, and (g) rosiglitazone interactions with JNK-3 (4KKH) using AutoDock Vina software

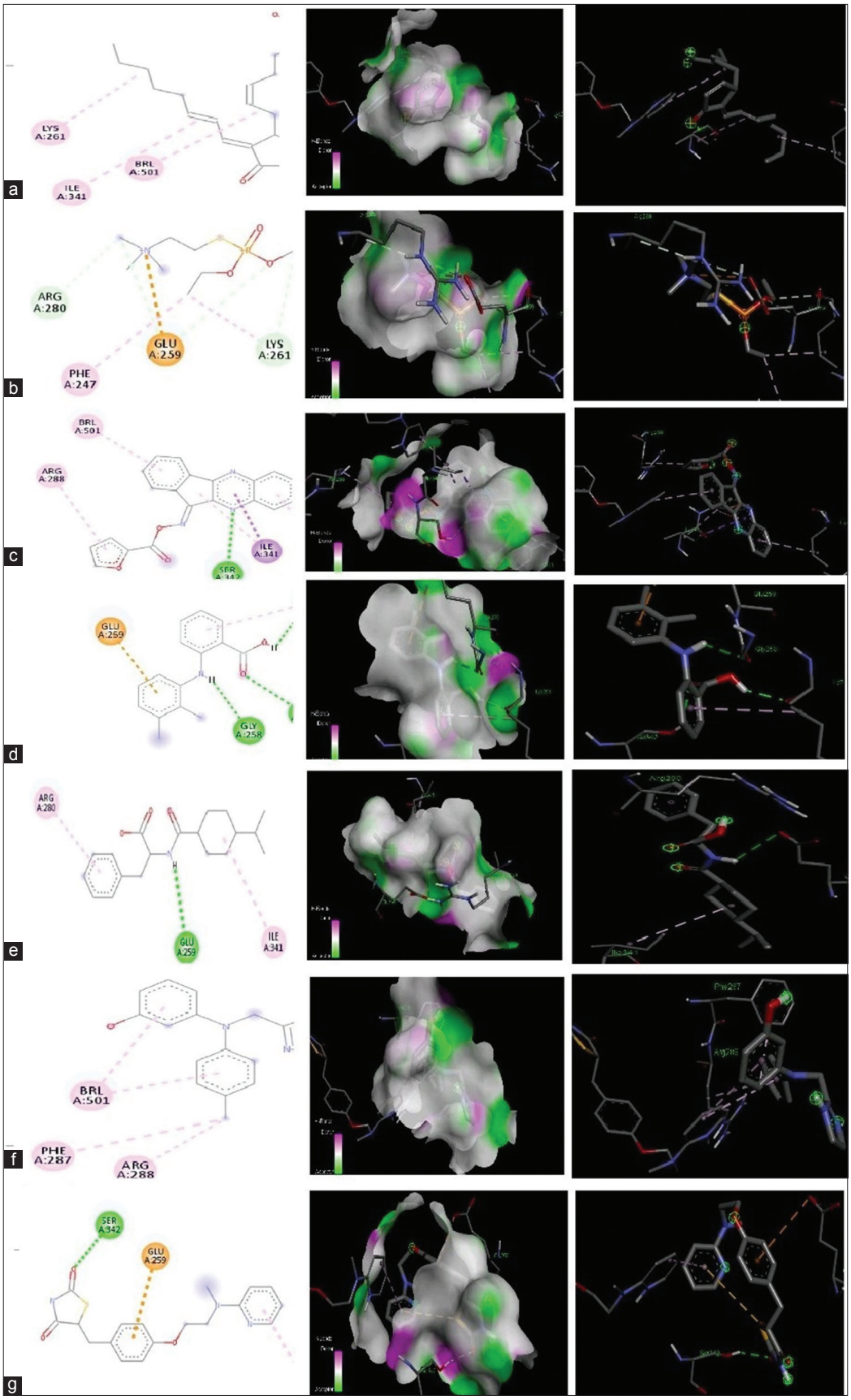

Figure 4: The two-dimensional and three-dimensional views of (a) 15d PGJ2, (b) echothiophate, (c) IQ3, (d) mefenamic acid, (e) nateglinide, (f) phentolamine (g) rosiglitazone interactions with PPAR gamma (PDBID:5YCP) using AutoDock Vina software

Table 5: Pharmacokinetic properties

Parameters	Echothiophate	Nateglinide	IQ3	15d-PGJ2	Phentolamine	Mefenamic acid
Absorption						
Water solubility (logmol/L)	–1.615	–2.996	–3.619	–5.013	–3.781	–2.93
Intestinal absorption (%)	93.563	100	98.226	95.215	94.045	96.888
Distribution						
VDss (human) (logL/kg)	0.161	–1.71	0.055	–0.75	0.612	–1.969
BBB permeability (logBB)	0.509	–0.064	–0.658	0.055	0.057	0.321
Metabolism						
CYP2D6 substrate	–	–	–	–	–	–
CYP3A4 substrate	–	–	+	+	+	–
CYP1A2 inhibitor	–	–	+	–	+	+
CYP2C19 inhibitor	–	–	+	–	+	–
CYP2C9 inhibitor	–	–	+	–	–	+
CYP2D6 inhibitor	–	–	–	–	+	–
CYP3A4 inhibitor	–	–	–	–	–	–
Excretion						
Total clearance (logml/min/kg)	0.225	1.282	0.809	1.789	0.379	0.334
Renal OCT2 substrate	–	–	–	–	+	–
Toxicity						
AMES toxicity	–	–	+	–	+	–
Hepatotoxicity	–	–	+	–	–	–

phentolamine [Figures 2-4]. The results of the toxic prediction suggest that all ligands are hepatotoxic free with the exception of ligand IQ3, and AMES toxicity was observed with IQ3 and phentolamine [Table 5].

CONCLUSION

In silico studies of ligands reduce cost, time in drug discovery and it'll reduce the failure of most medications in the clinical stage. This study has shown that these ligands have strong anti-AD properties against the four major AD targets. Among the six ligands evaluated, IQ3, nateglinide, phentolamine, and mefenamic acid significantly interacted with proteins but IQ3 and phentolamine had AMES toxicity. In summary, nateglinide and mefenamic acid need further evaluation as probable anti-Alzheimer's drugs considering the ADME parameters, toxicity, druglikeness, and docking scores. These tested molecules could be a good source of new drug development for AD.

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REFERENCES

1. Hayden EY, Eplow DB. Amyloid β-protein oligomers and Alzheimer's disease. Alzheimers Res Ther 2013;5:60.
2. Sanga Z, Wanga K, Zhangb P, Shia J, Liua W, Tan Z. Design, synthesis, *in silico* and biological evaluation of novel chalcone derivative sasmulti-function agents for the treatment of Alzheimer's disease. Eur J Med Chem 2019;180:238-52.
3. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: Prevalence estimates using the 2000 census. Arch Neurol 2003;60:1119-22.
4. Minati L, Edginton T, Bruzzone MG, Giaccone G. Reviews: Current concepts in Alzheimer's disease: A multidisciplinary review. Am J Alzheimers Dis Other Dement 2009;24:95-121.
5. Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. Mol Neuro Degen 2020;15:40.
6. Chaudhary A, Maurya PK, Yadav BS, Singh S, Mani A. Current therapeutic targets for Alzheimer's disease. J Biomed 2018;3:74-84.
7. Bekris LM, Yu CE, Tsuang DW. Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol 2010;23:213-27.
8. Kareti SR, Pharm SM. *In silico* molecular docking

analysis of potential anti-Alzheimer's compounds presentin chloroform extract of *Carissa carandas* leaf using gas chromatography MS/MS. Curr Ther Res 2020;93:100615.
9. Kumar P, Pillay V, Choonara YE, Modi G, Naidoo D, Toit LC. *In Silico* theoretical molecular modeling for Alzheimer's disease: The nicotine-curcumin paradigm in neuroprotection and neurotherapy. Int J Mol Sci 2011;12:694-724.
10. Takomthong P, Waiwut P, Yenjai C, Sripanidkulchai B, Reubroycharoen P, Lai R, *et al*. Structure-activity analysis and molecular docking studies of coumarins from *Toddalia asiatica* as multifunctional agents for Alzheimer's disease. Biomedicines 2020;8:107.
11. Mazumderand MK, Choudhury S. Tea polyphenolsas multi-target the rapeutics for Alzheimer's disease: An *in silico* study. Med Hypotheses 2019;125:94-9.
12. Mancinietal F. Beta-secretase as a target for Alzheimer's disease drug discovery: An over view of *in vitro* methods for characterization of inhibitors. Anal Bioanal Chem 2011;400:1979-96.
13. Muniyappa H, Das KC. Activation of c-Jun N-terminal kinase (JNK) by widely used specific p38 MAPK inhibitors SB202190 and SB203580: A MLK-3-MKK7-dependent mechanism. Cell Signal 2008;20:675-83.
14. Johnson GL, Nakamura K. The c-Jun kinase/stress-activated pathway: Regulation, function and role inhuman disease. Biochim Biophys Acta 2007;1773:1341-8.
15. Prajapat M, Sarma P, Shekhar N, Kaur H, Singh S, Kumar S, *et al*. *In silico* docking and comparative ADMET profile of different glycogen synthase kinase 3 beta inhibitors as the potential leads for the development of anti-Alzheimer drug therapy. J Adv Pharm Technol Res 2020;11:194-201.
16. Gourmaud S, Paquet C, Dumurgier J, Pace C, Bouras C, Gray F, *et al*. Increased levels of cerebrospinal fluid JNK3 associated with amyloid pathology: Links to cognitive decline. J Psychiatry Neurosci 2015;40:151-61.
17. Kothaetal S. An *in-silico* approach: Identification of PPAR-γ agonists from Seaweeds for the management of Alzheimer's disease. J Biomol Struct Dynam 2021;39:2210-9.
18. Landreth GE, Heneka MT. Anti-inflammatory actions of peroxisome proliferator-activated receptor gamma agonists in Alzheimer's disease. Neurobiol Aging 2001;22:937-44.
19. Landreth G, Jiang Q, Mandrekar S, Heneka M. PPAR agonists as therapeutics for the treatment of Alzheimer's disease. Neurotherapeutics 2008;5:481-9.
20. Hira S, Saleem U, Anwar F, Raza Z, Rehman AU, Ahmad B. *In silico* study and pharmacological evaluation of eplerinoneas an anti-Alzheimer's drug in STZ-induced Alzheimer's disease model. ACS Omega 2020;5:13973-83.
21. Kumar S, Chowdhury S, Kumar S. *In silico* repurposing of anti psychotic drugs for Alzheimer's disease. BMC Neurosci 2017;18:76.
22. Francis PT. The interplay of neurotransmitters in Alzheimer's disease. CNS Spectr 2005;10:6-9.
23. Grammas P, Ovase R. Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. Neurobiol Aging 2001;22:837-42.
24. Gannon M, Peng Y, Jiao K, Wang Q. The α2 adrenergic receptor as a novel target for Alzheimer's disease. FASEB J 2016;30:707.
25. Ricke KM, Cruz SA, Qin Z, Farrokhi K, Sharmin F, Zhang L, *et al*. Neuronal protein tyrosine phosphatase 1B hastens amyloid β-associated Alzheimer's disease in mice. J Neurosci 2020;40:1581-93.
26. Cheung J, Gary EN, Shiomi K, Rosenberry TL. Structures of human acetylcholinesterase bound to dihydrotanshinone I and territrem B show peripheral site flexibility.ACS Med Chem Lett 2013;4:1091-6.
27. Hong L, Koelsch G, Lin X, Wu S, Terzyan S, Ghosh AK, *et al*. Structure of the protease domain of memapsin 2(beta-secretase) complexed with inhibitor. Science 2000;290:150-3.
28. Yumietal E. Crystal Structures of Apo-and AMP-bound Human c-Jun N-Terminal Kinase 3. Bull Korean Chem Soc 2016;37:1360-3.
29. Jang JY, Bae H, Lee YJ, Choi YI, Kim HJ, Park SB, *et al*. Structural basis for the enhanced anti-diabetic efficacy of lobeglitazone on PPARγ. Sci Rep 2018;8:31.
30. Herawati H, Oktanella Y, Anisa AK. Molecular docking analysis of curcuminoids from *Curcuma longa* extract on iNOS as an immuno modulator candidate in broilers. Adv Anim Vet Sci 2021;9:519-24.
31. Foster DJ, Choi DL, Conn PJ, Rook JM. Activation of M1 and M4 muscarinic receptors as potential treatments for Alzheimer's disease and schizophrenia. Neuropsychiatr DisTreat 2014;10:183.
32. Pires DE, Blundell TL, Ascher DB. pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem 2015;58:4066.
33. Khare N, Maheshwari SK, Jha AK. Screening and identification of secondary metabolites in the bark of *Bauhinia variegata* to treat Alzheimer's disease by using molecular docking and molecular dynamics simulations. J Bimol Struct Dynam 2020;34:234-50.