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RESEARCH ARTICLE

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

Tirumalasetti Jaswitha¹, Chinthada Joshi²

¹Department of Pharmaceutics, Vallabhaneni Venkatadri Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh, India, ²Department of Pharmacology and Toxicology, Masters in Regulatory Toxicology, NIPER, Mohali, Punjab, India

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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

***Corresponding Author:** Tirumalasetti Jaswitha E-mail: jassujaswitha@gmail.com 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 AD, difficulty in remembering the recent events is most commonly seen early symptom. In most cases, people having disease those with the lateonset 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 proliferatoractivated 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 (A β) is generated by the endoproteolysis of A β 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 stressactivated 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 AB production, it can also raise BACE-1 expression.[14-16] Nuclear receptors such as PPAR-y is reported to play an important role in the lipid, glucose, and energy metabolism in the brain, it reduces the synthesis of A β , 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 several screening using 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 betaamyloid in senile plaques.^[12] Echothiophate is acetylcholinesterase acetylcholine inhibitor, levels are decreased in AD patients.^[22] 15d-PGJ2 is a PPAR-y 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 $\alpha 2$ adrenergic blocker, $\alpha 2$ adrenergic receptor activation enhances amyloidogenic processing of amyloid precursor protein (APP), results AB 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.

Table 1: Details of the receptors used in the study

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]

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 <i>et al</i> . ^[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
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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	$\mathrm{C_9H_{23}NO_3PS^{\scriptscriptstyle +}}$	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	$C_{17}H_{19}N_{3}O$	2	3	4	$\alpha 2$ 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

Jaswitha and Joshi: In silico pharmacokinetics and molecular docking of selected compounds against target proteins

Table 5. Drugiik	Table 5. Drughkeness violation of inguides								
Compound		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			

Table 3:	Druglikeness	violation	of ligand	s
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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

SN0 PBB ID Ligand Affmity (not) not Purpose of the solution of the	Table 4: Docking scores and amino acid interactions of ligands							
1. 400E 0,3 -9.0 HIS B:381, GLN A:527 HIS A:381, LEU B:300, ALA B:528, ARG B:525 1.54 PG127 -6.3 HIS B:381 HIS A:381, LEU B:300, EUA:350, THR A:383 1.54 PG127 -6.3 GLY A:523, GLN A:527 ARG A:525, IVS A:332, VAL A:429 1.64 Phentolimine acid -7.0 HIS A:405, ASNA A:533 PRO A:235, PRO A:410, GLU A:313 1.64 Phentolimine acid -8.0 HIS B:381, GLN A:527 HIS A:381 2.7 HFK R/3 -8.6 HIS B:381, GLN A:527 HIS A:302, PRO A:410, GLU A:313 2.8 HFK R/3 -8.6 HIS B:381, GLN A:527 HIS A:381, GLN A:528, PRO A:290, PRO A:368, LEU 2.4 HFK R/3 -8.6 LEU B:161, ARG B:307 PRO B:10, GLU B:301, TSY B:9, VAL B:309, PRO B:308 1.54 PG12 -4.1 ARG A:43P PS B:269, ASP B:317, TYR A:184, Pherotolimine acid -5.5 FIB E:27, TRP B:202 LYS B:246, LYS B:240, LYS B:246, LYS B:249, TYS B:256, VAL A:3, VAL A:48P 3.4 HKKH IQ3 -6.5 SER B:252 LYS B:249, LYS B:256, VAL A:3, VAL A:48P 4.5 MAGEJINA -4.5 SER B:257	S.No.	PDB ID	Ligand	Affinity (kcal/ mol)	Hydrogen bond	Residual hydrophobic/Pi-Cation/Pi-Anion/Pi-Alkyl interactions/Pi-Pi stacked interactions		
Echobiophete 154-P012 -5.3 HIS B:381 HIS A:381, LEU B:380, LEU A:380, THR A:383 154-P012 -6.3 GLY A:523, GLN A:527 NRG A:525, LYS A:332, VAL A:429 Photoloanina -7.0 HTR PA:532 NRG A:525, LYS A:332, VAL A:429 Photoloanina -7.0 HTR PA:532 NRG A:525, LYS A:332, VAL A:429 Donepezil -6.9 ASP A:400, GLN B:527 ALA:528, ARG A:525 -8.0 HIS B:381, GLN A:527 HIS A:381 -8.0 HIS B:381, GLN A:527 HIS A:381, RED A:290, ARG A:290, PRO A:368, LEU A:289, LEU A:540, PRO A:235, PRO A:410 -8.0 LEU B:161, ARG B:30 PRO B:160, GLU B:310, LYS B:9, VAL B:309, PRO B:308 LYS B:21 LYS B:226 YS B:249, LYS B:240, TYS B:249 Mefenamic acid -5.5 PHE B:257 CYS B:269, ASP B:317, TYR A:184, Mefenamic acid Mefenamic acid -5.6 SER B:252 LYS B:249, LYS B:256, LYS B:249, IYS B:249, LYS B:249, LYS B:256, LYS B:249, IYS B:256, VAL A:39, LYS A:33, ASI 34	1.	4M0E	IQ3	-9.0	HIS B:381, GLN A:527	HIS A:381, LEU B:380, ALA B:528, ARG B:525		
104-02 Metemanic acid Phentolamine Nateglinide Donepezil -6.3 GLY A-523, GLN A-527 ARG A:525, LYS A:332, VALA:429 11S A-405, ASN A.533, Phentolamine Donepezil -7.0 HIS A-405, ASN A.533, PRO A:235, PRO A:410, GLU A.313 2. 14K -6.9 ASP A:400, GLN B:527 ALA A:528, ARG A:525 -8.0 HIS B:381, GLN A:527 HIS A:381 -6.0 -6.0 ALA A:528, ARG A:290, ARG A:290, ARG A:290, PRO A:368, LEU A:540, PRO A:230, PRO A:230, PRO A:230, PRO B:308 154-PG12 -4.1 ARG A:43P CYS B:269, ASP B:317, TYR A:184, Metemanic acid Phentolamine Phentolamine -6.5 SER B:252 LYS B:256, VAL A:3, VAL A:48P Stemagacestat -6.5 SER B:257 CYS B:269, ASP B:317, TYR A:184, Metemanic acid Phentolamine -6.9 GLY B:158, GLN B:301, UB :364, TRP B:270 GLY B:273, PHE B:365 GLY B:273, PHE B:365 3. 4KKHI PQ3 -9.6 ARG A:107			Echothiophate	-5.3	HIS B:381	HIS A:381, LEU B:380, LEU A:380, THR A:383		
 Phenolamine Networking - 7.0 HIS A.405, ASN A.533, PRO A.235, PRO A.310, GLU A.313 TRP A.532 Phenolamine Nateginide Domepail -6.9 ASP A.400, GLN B.527 ALAA.528, ARG A.525 ALAA.528, ARG A.525 ALAA.528, ARG A.250, ARG A.296, PRO A.368, LEU -8.0 HIS B.381, GLN A.527 HIS A.208, PRO A.230, ARG A.296, PRO A.368, LEU -8.0 HIS B.381, GLN A.527 HIF A.238, PRO A.200, ARG A.296, PRO A.368, LEU -8.0 HIS B.261, ARG B.307 PRO B.160, GLU B.310, LYS B.9, VAL B.309, PRO B.308 LSV B.221 HFKN IQ3 -8.8 HIS A.406, A43P CYS B.260, ASP B.317, TYR A.184, Phenolamine acid Phenolamic acid -6.0 HIE B.257, TRP B.262 LYS B.256, ALA B.250, LYS B.246, LYS B.249 Phenolamic acid Phenolamic acid -6.5 HIS B.249 HIE B.257, TRP B.262 CYS B.249, LYS B.256, VAL A.3, VAL A.48P Semagacestat -6.5 HIS B.249, TYS B.246, LYS B.249, TRP B.262 Semagacestat -6.7 HIE B.257 HIE B.257 S B.249, LYS B.256, VAL A.3, VAL A.48P -6.8 HIS B.249, TYB B.256, VAL A.3, VAL A.48P -6.8 HIS B.249, TYB B.256, VAL A.3, VAL A.225, ARG A.230 LI B.34, TRP H.227 ALA A.74, LEU A.206, LYS A.93, VAL A.225, ARG A.230 HIS A.38 A.194 -6.7 HIE B.365 HIS A.38 A.194 -7.7 HIS A.39, ARG A.107 LI A.206, ASP A.207, GLU A.111 SAN A.194 -7.7 HIS A.93, ARG A.107 LI A.206, LYS A.93, ALA.74 -7.8 HIS A.310 -7.4 HIS A.310 -7.4 HIS A.310 -7.4 HIS A.310 -7.4 HIS A.310, HI			Mefenamic acid	-6.3	GLY A:523, GLN A:527	ARG A:525, LYS A:332, VAL A:429		
Donepezil -6.9 ASP A:400, GLN B:527 ALA A:528, ARG A:525 -8.0 HIS B:381, GLN A:527 HIS A:381 -8.6 ARG A:247 THR A:238, PRO A:290, ARG A:296, PRO A:368, LEU A:540, PRO A:210, ARG A:247 2. IFKN 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 LYS B:321 2. IFKN IQ3 -8.8 LEU B:161, ARG B:307, LYS B:324, LYS B:240, TRP B:262 Semagacestat -6.0 - VAL A:48P, VAL A:3, LYS B:256, LYS B:240, TRP B:262 Semagacestat -6.6 IYS B:240, TRP B:262, LYS B:240, LYS B:240, LYS B:240, LYS B:240, LYS B:240, LYS B:240, TRP B:262 Semagacestat -6.6 IYS B:240, TRP B:262, LYS B:240, TRP B:262, LYS B:240, TRP B:262, LYS B:240, LYS B:240, LYS B:240, LYS B:240, LYS B:240, LYS B:240, LYS B:240, TRP B:262, LYS B:240, TYS B:240, TRP B:262, LYS B:240, TYS B:240, LYS B:240, LYS B:240, LYS B:240, LYS A:33, VAL A:245, ARG A:230 3. 4KKH IQ3 -9.6 ARG A:107 LA:A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 3. -7.1 LYS A:93 S			Phentolamine Nateglinide	-7.0	HIS A:405, ASN A:533, TRP A:532	PRO A:235, PRO A:410, GLU A:313		
-8.0 HIS B:381, GLN A:327 HIS A:381 -8.6 AGG A:247 THR A:338, PRO A:290, ARG A:296, PRO A:368, LEU A:289, LEU A:540, PRO A:235, PRO A:410 2. IFKN 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 15.0+PG12 -4.1 AGA :43P CYS B:269, ASP B:317, TYR A:184, Mefenamic acid Mefenamic acid -6.0 VAL A:48P, VAL A:3, LYS B:246, LYS B:249 Nateglinide -6.0 - VAL A:48P, VAL A:3, LYS B:246, LYS B:249, TRP B:262 Semagacestat -6.5 SER B:252 LYS B:240, LYS B:240, LYS B:240, TYS B:249, TRP B:262 Semagacestat -6.6 LYB B:249, TRP B:262 VAL A:48P IVB B:249, ITP B:262 LYS B:249, LYS B:256, VAL A:3, VAL A:48P -6.8 LYB B:249, TRP B:363 GLY B:138, GLN B:303, GLY B:239, TYB A:239, ACA A:48P -6.8 LYB B:249, TRP B:365 CLU B:304, TRP B:261 VAL A:48P JME Echothiophate -6.7 LYS A:93, ARG A:107 LEU A:206, LYS A:93, VAL A:225, ARG A:230 JMEfenamic acid -7.1 LYS A:93, ARG A:107 LEU A:206, LYS A:93, VAL A:223 JMefenamic acid -7.5 ARG A:107 </th <td></td> <td></td> <td>Donepezil</td> <td>-6.9</td> <td>ASP A:400, GLN B:527</td> <td>ALA A:528, ARG A:525</td>			Donepezil	-6.9	ASP A:400, GLN B:527	ALA A:528, ARG A:525		
 IFKN IQ3 Echothiophate AFG A:247 THR A:238, PEO A:290, AEG A:296, PEO A:368, LEU A:289, LEU A:540, PRO A:235, PRO A:410 ARG A:289, LEU A:540, PRO A:235, PRO A:410 ARG A:289, LEU A:540, PRO A:235, PRO A:410 ARG A:499 CYS B:260, ALB B:201, LYS B:9, VAL B:309, PRO B:308 CYS B:260, ALB B:201, LYS B:240, LYS B:240 Mefenamic acid ARG A:49P CYS B:260, ALB B:201, LYS B:240, LYS B:249 Nateglinide ARG A:289, VAL A:3, UYS B:256, LYS B:249, TRP B:262 Semagacestat ARG A:290, TRP B:262 VAL A:48P, VAL A:3, UYS B:256, VAL A:3, VAL A:48P PHE B:257 Semagacestat ARG A:107 ALA :74, LEU A:206, LYS A:93, VAL A:225, ARG A:300 Echothiophate Set A:17, TYR A:184, Fechothiophate Set A:17, TYR A:184, Set A:17, TYR A:23 Set A:17, TYR A:23 Set A:17, TYR A:23, VAL A:225, ARG A:300 Echothiophate Set A:107 ALA :74, LEU A:206, LYS A:93, VAL A:225, ARG A:301 Echothiophate Set A:304, TYR B:257 Set A:17, VX A:93, ALA A:74, LEU A:206, LYS A:93, VAL A:223, ALA A:74 Set A:304 <l< th=""><td></td><td></td><td></td><td>-8.0</td><td>HIS B:381, GLN A:527</td><td>HIS A:381</td></l<>				-8.0	HIS B:381, GLN A:527	HIS A:381		
2. IFKN IQ3 -8.8 LEU B:161, ARG B:307, IYS B:321 PRO B:160, GLU B:310, LYS B:9, VAL B:309, PRO B:308 15G/FG12 -4.1 ARG A:3P CYS B:269, ASP B:317, TYR A:184, Mefenamic acid Phentolamine -5.5 PHE B:257, TRP B:262 LYS B:256, ALA B:250, LYS B:249, TRP B:262 Semagacestat -6.0 - VAL A:48P, VAL A:3, LYS B:256, LYS B:249, TRP B:262 -8.8 IYS B:249, TRP B:262, PHE B:257 VAL A:48P, VAL A:3, UYS B:256, UYA A:3, VAL A:48P -6.8 IYS B:249, TRP B:262, PHE B:257 VAL A:48P -6.8 IYS B:249, TRP B:262, PHE B:257 VAL A:48P -6.8 IYS B:249, TRP B:262, PHE B:257 VAL A:48P -6.8 IYS B:249, TRP B:262 VAL A:48P -6.8 IYS B:240, TRP B:262 VAL A:48P -6.8 IYS B:261, IYS B:250, VAL A:3, VAL A:28, IYS A:291 ALA A:74, LEU A:206, IYS A:93, VAL A:225, ARG A:230 -6.9 GLY B:158, GLN B:303, GLU B:30, IPA IYS A:93, AIG A:107 LEA :240, GASP A:207, GLU A:111 -15d-PG12 -7.1 LYS A:93, IYS A:191, AITefinitic acid AIA A:74, LEU A:261, IYS A:291, IEA :231, BRL A:301 -7.5 ARG A:107				-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		
154-PGJ2 -4.1 ARG A:43P CYS B:269, ASP B:317, TYR A:184, Mcfenamica acid Phentolamine -5.5 PHE B:257, TRP B:262 LYS B:256, ALA B:250, LYS B:249, TYS B:249 Nateglinide -6.0 - VAL A:48P, VAL A:3, IYS B:256, LYS B:249, TRP B:262 Semagacestat -6.5 SER B:252 LYS B:249, LYS B:256, VAL A:3, VAL A:48P -6.8 LYS B:249, TRP B:262, VAL A:48P VAL A:48P -6.8 LYS B:249, TRP B:262, VAL A:48P VAL A:48P -6.8 LYS B:257 VAL A:48P -6.9 GLY B:158, GLN B:303, GLU B:364, TRP B:277 GLY B:230, VAL A:225, ARG A:230 Semagacestat -6.9 ARG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 Sethothiophate -9.6 ARG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:223, ARG A:230 Mefenamic acid Phentolamine -6.5 LYS A:93, ARG A:101, ASN A:194 ALA A:74, LEU A:206, LYS A:93, VAL A:223, ALA A:74 SP600125 -7.1 LYS A:93 SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74 AF SYCP IQ3 -8.5 SER A:342 ARG A:280, IYS A:261, ILE A:341, BRL A:301 AF GLY -6.6 - LYS A:261, ILE A:341, BRL A:50	2.	1FKN	IQ3 Echothiophate	-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		
Michania add -5.5 PHE B:257, TRP B:262 LYS B:256, ALA B:250, LYS B:246, LYS B:249 Nateglinide -6.0 - VAL A:48P, VAL A:3, LYS B:256, LYS B:249, TRP B:262 Semagacestat -6.5 SER B:252 LYS B:249, LYS B:249, LYS B:249, TRP B:262 -6.8 LYS B:249, TRP B:262 VAL A:48P -6.8 LYS B:249, TRP B:262 VAL A:48P -6.9 GLY B:158, GLN B:303, GLY B:273, PHE B:365 GLY B:373, PHE B:365 -6.1 LYS A:93, ARG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 -15d-PG12 -4.7 LYS A:93, ARG A:107, AN:194 Elostoniophate -6.7 LYS A:93, LYS A:191, AN:194 ALA A:74, LEU A:206, ASP A:207, GLU A:111 Nateglinide -6.5 LYS A:93, LYS A:191, ASN A:194 ALA A:74, LEU A:206, ASP A:207, GLU A:211, ALA A:74 SP600125 -7.1 LYS A:93 SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74 -7.5 ARG A:107 LEV A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEV A:206, LYS A:93, ALA A:74 -7.7 - LYS A:93, ALA A:74, TYR A:223, ALA A:74 -7.6 ARG A:107 LEV A:2			15d-PGJ2 Mefenamic acid	-4.1	ARG A:43P	CYS B:269, ASP B:317, TYR A:184,		
Nateglinide Semagacestat -6.0 - VAL A:48P, VAL A:3, LYS B:256, LYS B:249, TRP B:262 -6.5 SER B:252 LYS B:249, TRP B:262, PHE B:257 VAL A:48P -6.8 LYS B:249, TRP B:262, PHE B:257 GLY B:273, PHE B:365 3. 4KH IQ3 -9.6 ARG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 5. 4KH IQ3 -9.6 ARG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 6. 4.7 LYS A:93, ARG A:107 ALA A:74, LEU A:206, ASP A:207, GLU A:111 15d-PGJ2 -4.7 LYS A:93, LYS A:194 ALA A:74, LEU A:95, TYR A:223, ALA A:74 Mefenamic acid -6.5 LYS A:93, LYS A:194 ALA A:74, LEU A:95, TYR A:223, ALA A:74 Phentolamine -6.5 LYS A:93 SER A:120 Nateglinide -7.1 LYS A:93 SER A:221 SP600125 -7.1 LYS A:93 SER A:220 -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 -7.7 -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 -6.6 SER A:342 ARG A:280, PHE A:247, GLY A:27			Phentolamine	-5.5	PHE B:257, TRP B:262	LYS B:256, ALA B:250, LYS B:246, LYS B:249		
Semagacestat -6.5 SER B:252 LYS B:249, LYS B:256, VAL A:3, VAL A:48P -6.8 LYS B:249, TRP B:262, PHE B:257 VAL A:48P -6.9 GLY B:158, GLN B:303, 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 3. 4KKH IQ3 -9.6 ARG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 4. Fchothiophate I:5d-PGI2 -4.7 LYS A:93, ARG A:107, ASN A:194 LEU A:206, ASP A:207, GLU A:111 Mefenamic acid Phentolamine Nateglinide -6.5 LYS A:93, LYS A:191, ASN A:194 ALA A:74, LEU A:95, TYR A:223, ALA A:74 5 YCP IQ3 -7.1 LYS A:93 SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74 4. 5 YCP IQ3 -7.1 LYS A:93 SER A:322 -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 -7.6 ARG A:107 LYS A:93, ALA A:74 4. 5 YCP IQ3 -8.5 SER A:342 ARG A:280, PHE A:247, GLU A:259, LEU A:310 4. SYCP IQ3 -6.6 SER A:342, GLY			Nateglinide	-6.0	-	VAL A:48P, VAL A:3, LYS B:256, LYS B:249, TRP B:262		
-6.8 LYS B:249, TRP B:262, PHE B:357 VAL A:48P PHE B:257 GLY B:158, GLN B:303, GLY B:273, PHE B:365 3. 4KKH IQ3 -9.6 AG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 3. 4KKH IQ3 -9.6 AGG A:107 ALA A:74, LEU A:206, LYS A:93, VAL A:225, ARG A:230 15d-PGJ2 -4.7 LYS A:93, ARG A:107, ASN A:194 ALA A:74, LEU A:95, TYR A:223 Phentolamine Atteglinide SP600125 -7.1 LYS A:93 SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74 5YCP IQ3 -7.5 ARG A:107 LYS A:93, ALA A:74, TYR A:223 4. 5YCP IQ3 -8.5 SER A:107 LEU A:206, LYS A:93, ALA A:74 4. 5YCP IQ3 -8.5 SER A:107 LEU A:206, LYS A:93, ALA A:74 4. 5YCP IQ3 -8.5 SER A:142 ARG A:107 LEU A:206, LYS A:93, ALA A:74 4. SYCP IQ3 -8.5 SER A:142 ARG A:280, PHE A:247, GLU A;259, LYS A:261 15d-PGJ2 -6.6 SER A:342 GLU A:259 LYS A:261, ILE A:341, BRL A:501 Phentolamine Attriphentic acid -6.5 SER A:342, GLY A:258			Semagacestat	-6.5	SER B:252	LYS B:249, LYS B:256, VAL A:3, VAL A:48P		
-6.9 GLY B:158, GLN B:303, 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 5. Echothiophate I5d-PGJ2 Mefenamic acid Phentolamine -4.7 LYS A:93, ARG A:107, ASN A:194 LEU A:206, ASP A:207, GLU A:111 ASN A:194 9. -4.7 LYS A:93, ARG A:107, ASN A:194 LEU A:206, ASP A:207, GLU A:111 ASN A:194 9. -4.7 LYS A:93, LYS A:191, ASN A:194 ALA A:74, LEU A:95, TYR A:223 9. -7.1 LYS A:93 SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74 9. -7.5 ARG A:107 LYS A:93, ALA A:74, TYR A:223 9. -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 9. -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 9. -7.6 ARG A:107 LYS A:93, ALA A:74 9. -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 9. -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 9. -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 9. -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 9. -7.6 SER A:3				-6.8	LYS B:249, TRP B:262, PHE B:257	VAL A:48P		
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Micronaline and Phentolamine -6.5 LYS A:93, LYS A:191, ASN A:194 ALA A:74, LEU A:95, TYR A:223 Nateglinide -7.1 LYS A:93 SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74 -7.5 ARG A:107 LYS A:106, TYR A:103, GLY A:209, LEU A:210, ALA A:214, ALA A:211 -7.7 - LYS A:93, ALA A:74, TYR A:223 -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 -7.6 ARG A:107 LEU A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEU A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEU A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEU A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEU A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEU A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEU A:206, LYS A:93, ALA A:74 -7.6 ARG A:107 LEU A:206, LYS A:261, ILE A:341, BRL A:301 -7.6 -8.5 SER A:342 ARG A:280, PHE A:247, GLU A;259, LYS A:261 I5d-PGJ2 -6.6 -7.5 GLU A:259 GLU A:259 Nateglinide -6.6 SER A:342, GLY A:258, BRL A:501 -7.5 -7.5 GLU A:259			Echothiophate 15d-PGJ2 Mefenamic acid Phentolamine Nateglinide SP600125	-4.7	LYS A:93, ARG A:107, ASN A:194	LEU A:206, ASP A:207, GLU A:111		
SP600125 -7.1 LYS A:93 SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74 -7.5 ARG A:107 LYS A:106, TYR A:103, GLY A:209, LEU A:210, ALA A:214, ALA A:211 -7.7 - LYS A:93, ALA A:74, TYR A:223 -7.6 ARG A:107 LYS A:93, ALA A:74, TYR A:223 -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 15d-PGJ2 -4.3 - ARG A:280, PHE A:247, GLU A;259, LYS A:261 Mefenamic acid Phentolamine Nateglinide Rosiglitazone -6.6 SER A:342, GLY A:258, LYS A:261 GLU A:259 -7.5 GLU A:259 ILE A:341, ARG A:280 -7.5 -7.5 GLU A:259 ILE A:341, ARG A:280				-6.5	LYS A:93, LYS A:191, ASN A:194	ALA A:74, LEU A:95, TYR A:223		
-7.5 ARG A:107 LYS A:106, TYR A:103, GLY A:209, LEU A:210, ALA A:211, ALA A:211 -7.6 -7.7 - LYS A:93, ALA A:74, TYR A:223 -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, GLU A:259 GLU A:259 Nateglinide -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -7.5 GLU A:259, BRL A:501 ILE A:341, ARG A:280				-7.1	LYS A:93	SER A:72, VAL A:78, GLY A:73, TYR A:223, ALA A:74		
-7.7 - LYS A:93, ALA A:74, TYR A:223 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 I5d-PGJ2 -6.3 - LYS A:261, ILE A:341, BRL A:501 Phentolamine -6.6 SER A:342, GLY A:258, LYS A:261 LYS A:259 Nateglinide -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501				-7.5	ARG A:107	LYS A:106, TYR A:103, GLY A:209, LEU A:210, ALA A:214, ALA A:211		
4. 5YCP IQ3 -8.5 SER A:342 ARG A:288, LYS A:261, ILE A:341, BRL A:301 5YCP IQ3 -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 Phentolamine Acid -6.6 SER A:342, GLY A:258, LYS A:261 LYS A:261, ILE A:341, BRL A:501 Rosiglitazone -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501				-7.7	-	LYS A:93, ALA A:74, TYR A:223		
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 I5d-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 Nateglinide -6.6 - ARG A:288, PHE A:287, BRL A:501 Rosiglitazone -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501				-7.6	ARG A:107	LEU A:206, LYS A:93, ALA A:74		
Echothiophate 15d-PGJ2 -4.3 - ARG A:280, PHE A:247, GLU A;259, LYS A:261 Mefenamic acid -6.3 - LYS A:261, ILE A:341, BRL A:501 Phentolamine Nateglinide -6.6 SER A:342, GLY A:258, LYS A:261 GLU A:259 Rosiglitazone -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501	4.	5YCP	IQ3	-8.5	SER A:342	ARG A:288, LYS A:261, ILE A:341, BRL A:301		
Mefenamic acid -6.3 - LYS A:261, ILE A:341, BRL A:501 Phentolamine -6.6 SER A:342, GLY A:258, LYS A:261 GLU A:259 Nateglinide LYS A:261 -6.6 - Rosiglitazone -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501			Echothiophate	-4.3	-	ARG A:280, PHE A:247, GLU A;259, LYS A:261		
Phentolamine Nateglinide -6.6 SER A:342, GLY A:258, LYS A:261 GLU A:259 Rosiglitazone -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501			Mefenamic acid	-6.3	-	LYS A:261, ILE A:341, BRL A:501		
Rosiglitazone -6.6 - ARG A:288, PHE A:287, BRL A:501 -7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501			Phentolamine Nateglinide Rosiglitazone	-6.6	SER A:342, GLY A:258, LYS A:261	GLU A:259		
-7.5 GLU A:259 ILE A:341, ARG A:280 -6.6 SER A:342 GLU A:259, BRL A:501				-6.6	-	ARG A:288, PHE A:287, BRL A:501		
-6.6 SER A:342 GLU A:259, BRL A:501				-7.5	GLU A:259	ILE A:341, ARG A:280		
				-6.6	SER A:342	GLU A:259, BRL A:501		

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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 octanolwater 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	_	_	+	_	_	_		

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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IJPBA/Apr-Jun-2021/Vol 12/Issue 2

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