- RESEARCH ARTICLE-

The Interaction of Ile-Phe Dipeptide with Phosphatidylinositide 3-Kinase (PI3K): Molecular Dynamics and Molecular Docking Studies

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Abstract

Self-assembly of phenylalanine creates Phe fibers which is the characteristic fiber model found in amyloid fibrils linked with various neurodegenerative diseases. L-Isoleucyl-L-Phenylalanine (Ile-Phe) dipeptide is composed of isoleucine and phenylalanine amino acids, having fibrillary structure similar to nanotube forms of the core recognition motif of Alzheimer's β-amyloid polypeptide. The integrated coordination of neuronal responses via the PI3-K / Akt pathway has a significant functional impact on Alzheimer's disease. Exposure to Aβ in neuronal cultures leads to deterioration of PI3-K, Akt and mTOR signaling, which may cause cognitive loss during disease. Modulation of PI3-K, Akt and mTOR signal activity need aim to reduce or eliminate the accumulation of potential neurotoxins. The therapeutic approaches aimed to normalize neuronal responses on these pathways or activation of PI3-kinase have a protective effect against cognitive decline in animal models of Alzheimer disease. This study aims to determine the interaction of PI3-kinase with Ile-Phe dipeptide having amyloid fibril structure by three-dimensional simulation techniques such as molecular dynamics (with the GROMACS program) and molecular docking techniques (Schrodinger Software program).

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Introduction

Protein misfolding and accumulation of amyloid aggregates are the leading causes of a wide variety of diseases including multiple neurodegenerative disorders such as Alzheimer's disease (Wolfe & Cyr, 2011). In Alzheimer's disease, the fibers which are known as an amyloid fibers form amyloid plaques in the brain and the main component of these plaques are made of a molecule known as amyloid-beta. Amyloid-β (Aβ) in the brain tissues and cerebral vessels of Alzheimer's patients accumulates as plaques, leading to impaired neurovascular function and chronic neurodegeneration also have numerous toxic effects on neurons, astrocytes, glial cells and brain endothelium (Turkseven, 2014). The Phe-Phe (L-Phenylalanyl-L-Phenylalanine) dipeptide having create nanotube form, relates to residues 19 and 20 of the central hydrophobic cluster of the highly amyloidogenic peptide Aβ42 (Görbitz, 2006; Kol et al., 2005). The Ile-Phe dipeptide, which consist of Phe (phenylalanine) residue, having fibrillar structure form are similar to nanotube forms that the core recognition motif (Phe-Phe) of β-amyloid polypeptide (de Groot et al., 2007).

PI3K (Phosphoinositide 3-kinase) plays important role for mediating neuronal survival under different circumstances. The PI3K / Akt pathway functions as neuroprotective agents that inhibit neuronal apoptosis (Kitagishi et al., 2014). The pathways of PI3K aid to the survival of cells under certain conditions (Brunet et al., 2001; Xu et al., 2005). Defective Aβ protein metabolism and many different biochemical pathways including inflammatory, oxidative and hormonal pathways can trigger Alzheimer's disease. The evaluation of all these pathways has a significant potential for disease prevention and treatment. (Kitagishi et al., 2014).

In this study, conformational changes of Ile-Phe dipeptide in aqueous medium were investigated by using MD method and its interaction with PI3K/Akt receptor was determined by molecular docking method. The aim of this study is to elucidate by using the molecular docking technique the mechanism of interaction between Ile-Phe dipeptide which is an amyloid-like peptide molecule structure and PI3K/Akt receptor for better understanding the pathogenesis of Alzheimer's disease.

Material and Methods

Molecular Dynamic (MD) Simulation

The molecular dynamic simulation was realized with GROMACS 5.2 version (Van Der Spoel et al., 2005) using GROMOS96 43a1 force field (van Gunsteren et al., 1996). The structure of Ile-Phe which was optimized in the Gaussian 09 software program (Frisch et al., 2009) at DFT/B3LYP level of theory with the 6-31++G(d,p) basis set was used for the molecular dynamic simulation. The starting structure of the peptide was placed into a cubic box of SPC (simple point charge) (Smith & van Gunsteren, 1993) water molecules. Na+ and CI- ions added to box for system neutralization. After the neutral system was adjusted, energy was minimized by the steepest descent algorithm.The energy-minimized structure was balanced with NVT and NPT ensembles using Leapfrog algorithm to stabilize the temperature and pressure at 310 K and 1 bar, respectively. NVT and NPT systems are important for the preparation of the system in accordance with the experimental environment such as equilibration at a certain temperature or pressure communities. NVT calculation was achieved with a 2 fs time step for 25,000 steps and temperature coupling is applied using the Berendsen method (Berendsen, 1991). NPT calculation was executed for 250,000 steps with a 2 fs time step. In addition, the Parrinello-Rahman method (Parrinello and Rahman,

1981) was used to generate isotropically 1.0 bar pressure. After calculations of NVT and NPT, the molecular dynamic calculation was achieved for 5 ns with a 2 fs time step. LINCS algorithm (Hess et al., 1997) was applied to all bonds containing hydrogen bonds. All graphics of molecular dynamic calculation of Ile-Phe were plotted using XMGRACE (Turner, 2005).

Molecular Docking and ADME Properties

All computations like LigPrep, prepwizard, grid generation and docking in the molecular docking study was implemented by Schrodinger Maestro software using the Glide SP module (Friesner et al., 2004; Friesner et al., 2006; Halgren et al., 2004). As the first step, Ile-Phe was prepared by LigPrep tool using the OPLS force field as a ligand (Harder et al., 2015). PI3 kinase gamma (Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma isoform) which has 966 sequence length (PDB code: 3MJW) was selected as a receptor. PI3 kinase gamma is an enzyme encoded by the PIK3CG gene in humans. Phosphoinositol 3-kinase (PI3K) is important because it is associated with cell growth, mitogenic signaling, cell survival, proliferation and metabolic control (Zhang et al., 2010).The structure of receptor was obtained using SWISS-MODEL (Bienert et al., 2016). The target receptor was prepared for docking calculation with Protein Preparation Wizard tool (Sastry et al., 2013). In the system, water molecules and ions were extracted, polar hydrogen atoms were annexed to receptor. Also, bond orders were appointed, charges were described using PROPKA (Sondergaard et al., 2011) at neutral pH, and receptor was optimized. Energy minimization was implemented by preferring 0.3 Å RMSD and the OPLS3 force field to converge heavy atoms in the process of preparing the receptor. A grid box was generated in the active site defining mobile groups of receptor using grid generation tool of Glide module. The aim of grid generation was to determine possible ligand protein binding areas for ligand-receptor docking. After all preparations, Ile-Phe was docked into the receptor using SP (standard precision) algorithm. Absorption, distribution, metabolism, and excretion (ADME) properties, widely used in drug discovery, were also obtained using the Qik-Prop module of the Schrodinger software (Release, 2017). These pharmacokinetic properties are important for drug studies like octanol–water partition coefficient, brain/blood partition coefficient, and aqueous solubility.

Results

Molecular Dynamic Simulation Results

The initial structure of the Ile-Phe dipeptide was formed by using an optimum geometry into a cubic box of SPC (simple point charge) water molecules with 842 water molecules. Counter ions ($Na^+(2)$) and ($Cl^-(2)$) were filled to neutralize system, as shown Figure 1.
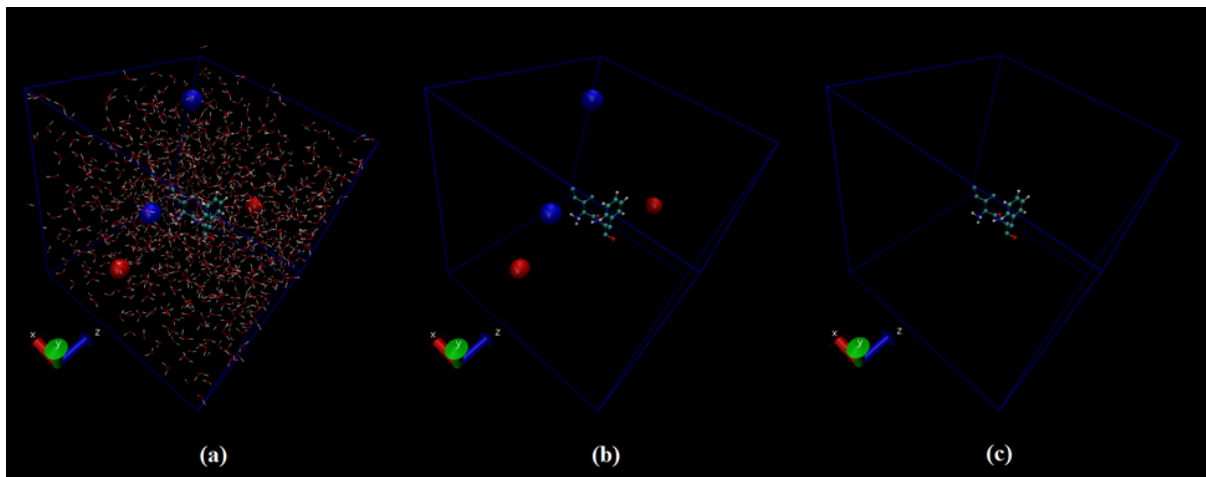
Figure 1. The beginning conformation of the Ile-Phe with 842 water molecules and 2 Na+ and 2 CI- ions in a cubic box (a), Ile-Phe with 2 Na+ and 2 CI- ions in a cubic box (b), lle-Phe in cubic box (c).

Using steepest descent algorithm, the energy minimization was implemented for 172 ps with -4.1968781x 104 kJ/mol potential energy, as shown Figure 2a. After minimization step, system was stabilized with NVT and NPT ensembles using Leapfrog algorithm at 310 K and 1 bar. NVT calculation was executed for 25,000 steps and temperature coupling is performed using the Berendsen method. NVT results were showed that system is well balanced around 310 K in Figure 2b. NPT calculation was achieved 250,000 steps with a 2 fs time step. The Parrinello-Rahman method was also used to couple pressure isotropically to a value of 1.0 bar. The NPT Simulation notified a density of 979.411 kg/m^3 as averaged of the 500 ps of simulation in Figure 3. MD simulations were actualized with GROMOS96 43a1 force field. MD simulation for 2,500,000 MD steps, totaling 5 ns of system was implemented. According to MD simulation results, total, kinetic and potential energy were obtained as -3.02753x10^4 kJ/mol, 6.59308x10^3 kJ/mol and -3.68684x10^4 kJ/mol, respectively. These energy graphics were shown in Figure 4.
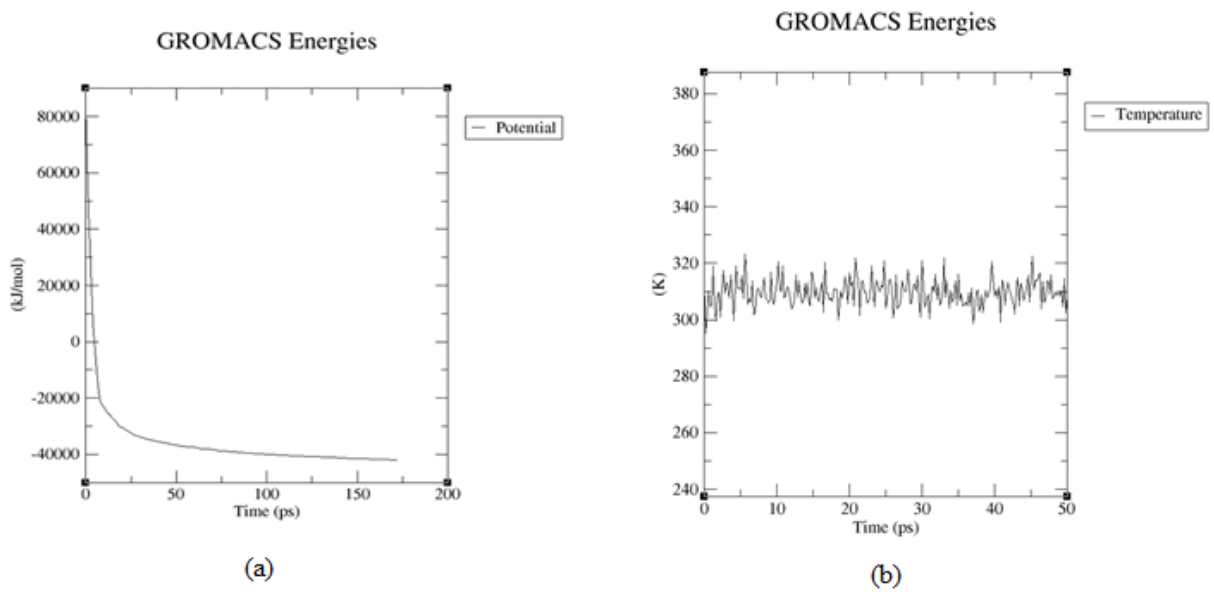
Figure 2. The potential energy of the system as a function of the minimization step using Steepest Descent algorithm (a), the equilibrated temperature of the system around 310 K (b).
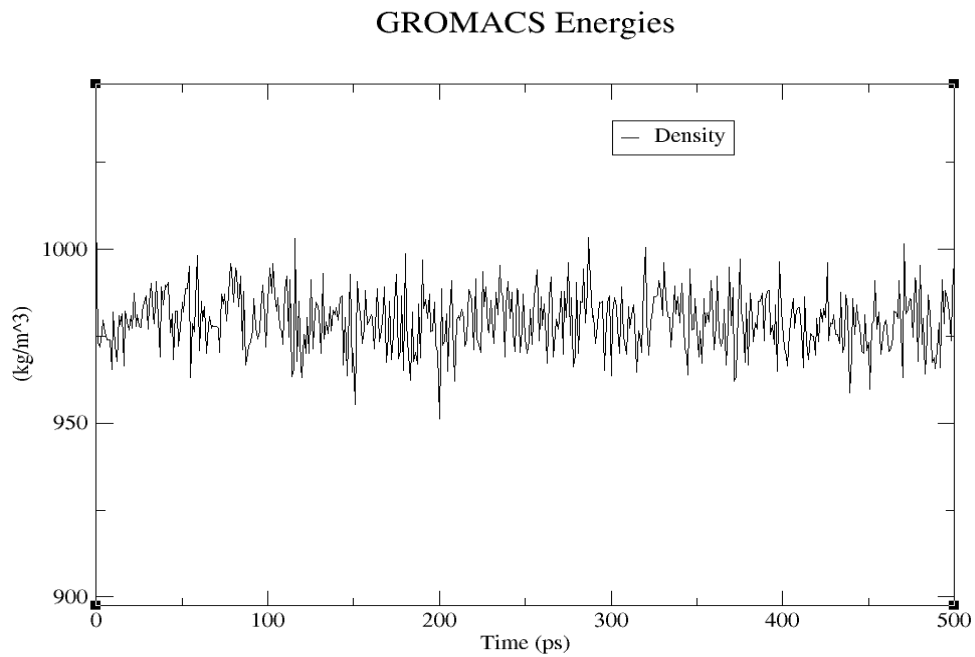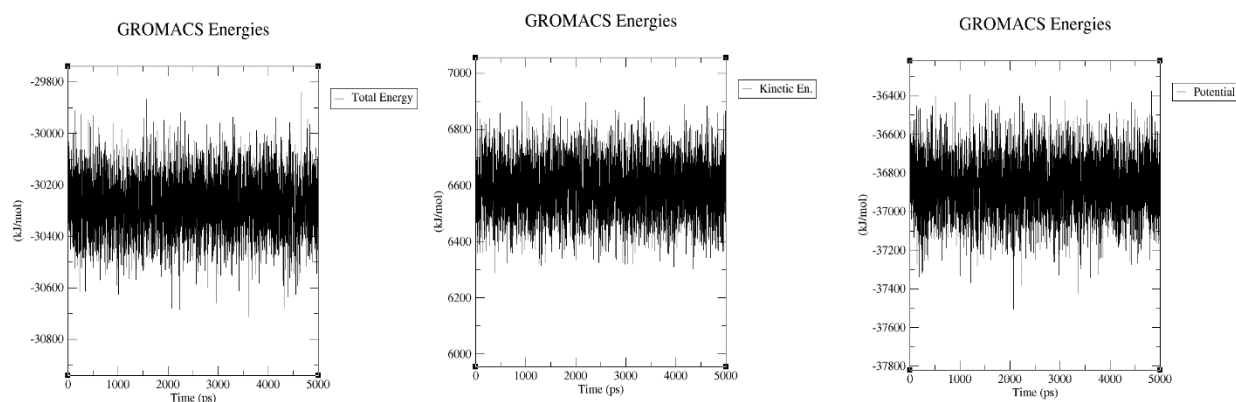

Figure 3. The density of system.

Figure 4. The total energy, kinetic energy and potential energy of the system.

After MD simulation, Root Mean Square Deviation (RMSD) and Radius of Gyration (Rg) were calculated. The range of RMSD was seen under 0.1 nm. Gyrate shows the compactness of each molecule. Rg value changed from 0.338468 to 0.361872 for 5000 ps. RMSD and Rg graphics were shown in Figure 5.
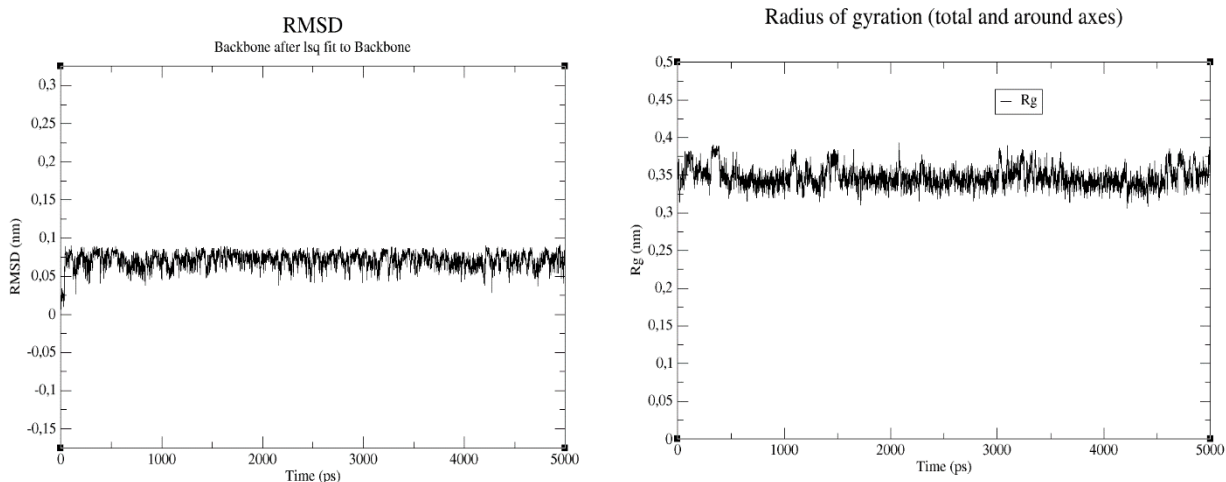
Figure 5. The RMSD values and radius of gyration of the system.

Molecular Docking and ADME Results

Molecular docking can be applied to identify the ligand binding pocket and to predict the interactions between ligand and receptor at the atomic level (Wodak & Janin, 1978). The conformation and docking score energies were listed in Table 1. In all these binding poses, the most stable binding energy was found to be -6.645 kcal/mol in Figure 6.

Table 1. The conformation and docking score energies

Ligand	Energies of the Ligand (kcal/mol)	Docking Score (kcal/mol)
1	**28.815**	**-6.645**
2	28.907	-6.621
3	25.194	-6.586
4	25.436	-6.485
5	25.062	-6.370
6	25.130	-6.314
7	28.827	-6.287
8	29.049	-6.238
9	34.486	-5.192
10	31.540	-5.097
11	30.483	-5.088
12	31.477	-5.088
13	32.498	-5.009
14	34.750	-4.656
15	30.210	-4.413
16	34.470	-4.308

Figure 6. The 3D image of interaction of Ile-Phe and Phosphatidylinositide 3-Kinase (PI3K).

The binding regions with hydrogen bonds of Ile-Phe to the PI3 kinase gamma are LYS691 and ASP808 residues and these binding regions and close interactions were shown in Figure 7a and Figure 7b. The O atoms in the carboxyl group of Ile-Phe and H atoms in amino group of Ile-Phe were linked to the positive charged amino acid LYS691 with 1.61 Å H-bond length and the negative charged amino acid ASP808 with 2.19 Å H-bond length, respectively. Additionally, it was seen that the molecule also makes salt bridges in regions of hydrogen bonding, shown in Figure 7b with red and blue line. Salt bridges occur from a combination of hydrogen bonding and ionic bonding.
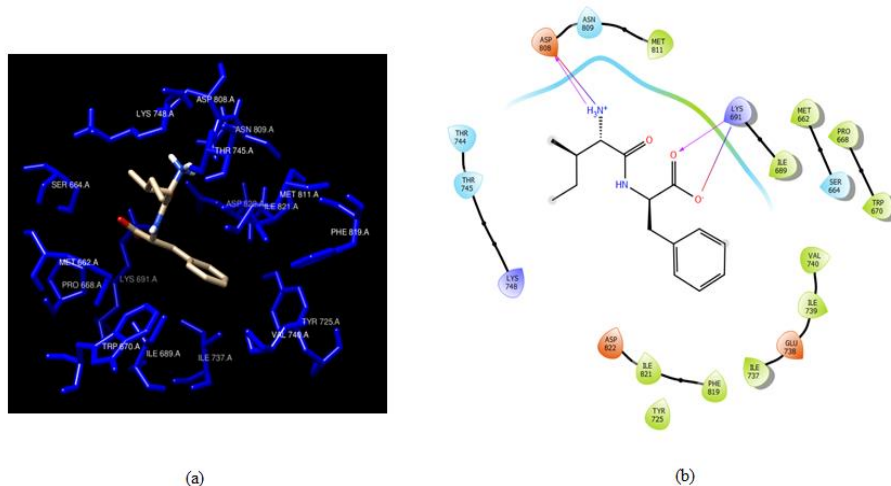

Figure 7. The close interactions of Ile-Phe and Phosphatidylinositide 3-Kinase (PI3K) (a), 2D ligand interaction in the active side of the Phosphatidylinositide 3-Kinase (PI3K) (b).

The electrostatic potential map surfaces shown in Figure 8 of the Ile-Phe and receptor enzyme were also formed to define the regions that were electron-rich and electron-poor. The surfaces are shown as the lowest electrostatic potential energy value in red and the highest in dark blue.
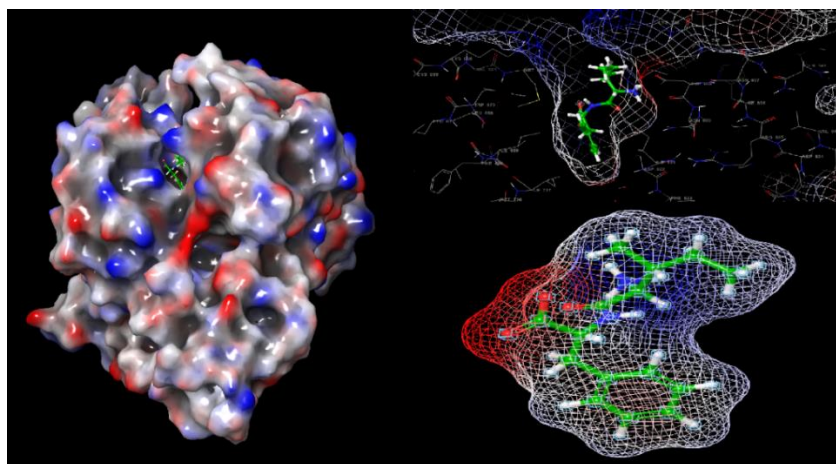

Figure 8. The electrostatic potential of Ile-Phe and Phosphatidylinositide 3-Kinase (PI3K).

ADME profiles were also determined for the interaction of dipeptide with different tissues in the body and to reveal the performance of this interaction. ADME profile was determined using Qikprop tool in the Schrodinger software. Molecular weight (mol_MW), hydrogen bond donors and acceptors and octanol-water partition coefficient (logPo/w) are properties based on Lipinski 5 rules. These properties for Ile-Phe molecule were determined as 278.350 g/mol, 3.25, 4.75 and -0.337, respectively (cf. Table-2). With the data obtained, it is seen that the molecule complies with Lipinski 5 rules. Human serum albumin (HSA) is as effective as the blood-brain barrier. Interactions of HSA and small molecules affect the ADME properties which calculated for small molecules. The calculated ADME properties of small molecules were affected by the interactions of HSA (Benet et al., 1996; Lexa et al., 2014). QP log K hsa Serum Protein Binding value was defined as -0.595 (standard limits from -1.5 to 1.5). While the blood-brain barrier allows of diffusion of small molecules having polar and hydrophobic properties, it obstructs the dissolution of large and hydrophilic molecules in the cerebrospinal fluid (CSF) (Johansen et al., 2018). The ability of pass through the blood–brain barrier was given with QPlogBB parameter, QPlogBB (Brain/blood partition coefficient) parameter was obtained as -0.898 (standard limits from -3.0 to 1.2). The values of apparent Caco-2 (human colonic adenocarcinoma cell) permeability and apparent MDCK (Madin Darby Canine Kidney) permeability is considered and the Caco-2 permeability (nm/sec) and MDCK permeability (nm/sec) were obtained as 15 and 11, respectively. These results showed that Caco-2 and MDCK permeabilities are weak. Additionally, The QP log Kp for skin permeability parameter is also significant. Computed skin permeability value was -4.965. Human oral absorption was also calculated as %46 (<25% is poor), as a result of work with Qikprop tool.

Table 2. Docking score and calculated ADME properties.

Property	Value	Recommended (Release, 2017)
Docking score (kcal/mol)	-6.645	
Polar surface area PSA (A˚2)	100.240	(7.0 / 200.0)
Molecular weight MW (g/mol)	278.350	(130.0 / 725.0)
Solute as Donor-Hydrogen Bonds	3.250	(0.0/ 6.0)
Solute as Acceptor-Hydrogen Bonds	4.750	(2.0/ 20.0)
Solute Ionization Potential (eV)	9.421	(7.9/ 10.5)
Solute Electron Affinity (eV)	-0.243	(-0.9/ 1.7)
Polarizability (Angstroms^3)	29.163M	(13.0 / 70.0)
QP log P for hexadecane/gas	10.276M	(4.0 / 18.0)
QP log P for octanol/gas	17.459M	(8.0 / 35.0)
QP log P for water/gas	12.643M	(4.0 / 45.0)
QP log P for octanol/water	-0.337	(-2.0 / 6.5)
QP log S for aqueous solubility	-1.860	(-6.5 / 0.5)
QP log S - conformation independent	-1.537	(-6.5 / 0.5)
QP log K hsa Serum Protein Binding	-0.595	(-1.5 / 1.5)
QP log BB for brain/blood	-0.898	(-3.0 / 1.2)

No. of Primary Metabolites	5	(1.0 / 8.0)
Predicted CNS Activity (-- to ++)	--	
HERG K+ Channel Blockage: log IC50	-2.284	(concern below -5)
Apparent Caco-2 Permeability (nm/sec)	15	(<25 poor. >500 great)
Apparent MDCK Permeability (nm/sec)	11	(<25 poor. >500 great)
QP log Kp for skin permeability	-4.965	(Kp in cm/hr)
Jm. max transdermal transport rate	0.042	(micrograms/cm^2-hr)
Lipinski Rule of 5 Violations	0	(maximum is 4)
Jorgensen Rule of 3 Violations	1	(maximum is 3)
% Human Oral Absorption in GI (+-20%)	46	(<25% is poor)

Conclusions

In this study, the conformational change of Ile-Phe dipeptide in aqueous medium was investigated by molecular dynamics method and the interaction between dipeptide having amyloid fibril structure and PI3-kinase enzyme, which is important for the treatment of neurodegenerative diseases, was demonstrated by using molecular docking technique for the first time. The O atoms in the carboxyl group and H atoms in amino group of Ile-Phe were bonded the positive charged amino acid LYS691 and the negative charged amino acid ASP808 by H-bonds, respectively. Salt bridges occur from a combination of hydrogen bonding and ionic bonding with same residues in the active side of the enzyme. Also, the pharmacokinetics and pharmacology profiles such as oral availability, brain barrier, molecular weight, and octonal water ratio coefficients of title dipeptide have also been demonstrated for the first time in our study.

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Conflict of Interest: No potential conflict of interest was reported by the authors.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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