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In Silico Study Of Four Alkaloids As Dipeptidyl Peptidase-4 (Dpp4) Inhibitors To Generate Anti-Diabetics Effect.

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Abstract

Diabetes is among the most prevalent diseases around the globe and the number of diabetics has been increasing at an alarming rate. Several drug targets have been explored and various drugs have been studied and developed to combat type-2 variation of the disease. The focus of drug development has been now shifted to natural products due to this urgent need in the current scenario. Among them, several flavonoids have been studied for their anti-diabetic potential extensively. In the present study, we tested the potentiality of four compounds targeting dipeptidyl peptidase-4 (DPP4), a type II transmembrane glycoprotein that play a vital role in the metabolism of glucose. DPP-4 inhibitors reduce glucagon and blood glucose levels. The mechanism of DPP-4 inhibitors is to increase incretin levels (GLP-1 and GIP),which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels. We performed molecular docking analysis and tested the stability of the complexes using molecular dynamics simulations and explored their pharmacokinetic properties. The binding energy of the compounds ranged from -6.8 to -7.1 Kcal/mol. Further, the post molecular dynamics (MD) analysis showed that all of them were greatly stable with DPP4 having similar results. Pharmacokinetic parameters of the compounds revealed very good properties in terms of adsorption, distribution, metabolism and excretion. Our study showed that these four compounds may turn out to be potent in treating malfunctioning of DPP4 to maintain glucose levels.

Keyword : Diabetes mellitus (DM), Dipeptidyl peptidase-4 (DPP4), alkaloids

1. Introduction

Diabetes mellitus (DM) is keep increasing and being leading worldwide health issue; especially type 2 DM [1]. The prevalence of diabetes mellitus (DM) is growing globally and it has been estimated to touch 642 million marks by the end of 2040 (IDF-Diabetes Atlas 8thedn). It will be a serious health issue in the near future because of an increase in the number of diabetes patients at an alarming rate [2]. Due to its various adverse effects worsening clinical conditions, the existing therapy for the treatment of type 2 DM is not sufficient [3].

So, there is an urgent need to find out novel breakthrough natural medicines due to the limitations of the currently available regimen for diabetes. The use of an integrated approach for the identification of novel inhibitors against a validated protein might be potentially desirable in this context.

Among several proteins to target treatment for diabetes mellitus, dipeptidyl peptidase-4 (DPP4) protein is encoded by the DPP4 gene which is a membrane serine exopeptidase that functions by cleaving X-proline dipeptides from the N-terminus of polypeptides [4]. DPP4 is a serine protease that breaks down peptidic hormone glucagon-like peptide 1 (GLP-1) which plays a significant role in regulating insulin release thereby controlling the level of blood glucose in human body [5,6]. Various studies demonstrate that the inhibition of DPP4 could play a role in increasing incretin levels (GLP-1 and GIP),which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose [7,8]. Therefore, it has considered a promising target for developing a novel drug for the treatment of type 2 diabetes.

Drug design and discovery are driven by advances

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in powerful cheminformatics and various in silico tools culminated in the revolution of 'bioinformatics era' [9]. The in-silico approach uses the combination of concepts of computer algorithms and statistical methods with advancements in high-performance computation in biological science [10]. Therefore, bioinformatics and computational biology has become the central paradigm for speeding up the process and cost of probing new potential candidates for any targeted disease [11]. Two major approaches i.e., structure and ligand based virtual screening approaches have extensively been used to discover lead compounds despite having some limitations in them [12]. Integration of these in silico techniques seems to be a reliable approach in identifying potential candidates with improved therapeutic uses.

Since ancient times, some herbal remedies have been used to cure diabetes, manage blood sugar levels, and prevent insulin resistance, which is the leading cause of type 2 diabetes, [13]. Medicinal plants are distinguished by the presence of secondary metabolites such as alkaloids which showed hypoglycemic activity [14]. Four alkaloids isolated from Lupinus hirsutus Linn. and Sophora secundiflora [15] were selected for identifying their potential efficacy against DPP4 protein in this study.

2. Materials and methods

2.1. Preparation of compounds and receptor

Four compounds named cytisine, N-methyl-cytisine, epilupipine and epilupipine-N-oxide were selected for this study. The 3D structures of the compounds selected were drawn in ChemDraw Ultra 12.0 [16] and their canonical SMILES were generated. Then they were converted into protein data bank files (PDB) using Online SMILES Translator and Structure File Generator (https://cactus.nci.nih.gov/translate/). The crystal structure of human dipeptidyl peptidase IV (DPP4) in complex with a decapeptide (tNPY) at 2.3 Å resolution was available in Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) [17]. The structure was collected and all the ligand molecules, hetero atoms and water molecules were removed from the structure using Discovery Studio 4.0 client (http://accelrys.com/products/discovery-studio/) [18].

2.2. Molecular docking simulation of four compounds with DPP4

Molecular docking was performed using PyRX [19], an open-source virtual screening software to determine receptor-ligand interactions. The important catalytic site residues (Arg125, Glu205, Glu206, Phe357, Tyr547, Ser630, Tyr631, Asn710 and His740) were enclosed with the parameters of grid box with X=23, Y=23, Z=23 (Center grid box: X = 60.331, Y = 10.448, Z = 21.064) dimensions. AutoDock Vina (ADV) [20], implemented in PyRX was used to accomplish all the docking simulations with the predetermined parameters. Further, the protein-ligand interaction was visualized by the Discovery studio 4.0 client and Pymol 1.1 [21].

2.3. Molecular dynamics (MD) simulation analysis

Molecular dynamics simulation of the four protein-ligand complexes was performed using GROMACS 2021.1 [22] version and Linux 5.4 package. The GROMOS96 54a7 [23] forcefield was used as the force field for proteins and the ligand topologies were generated from PRODRG [24] server. All the protein-ligand complexes were solvated using simple point charge (SPC) water molecules in a rectangular box. The required number of Na+ and Cl- ions were added while 0.15 mol/L salt concentrations were set in all the systems to make the simulation system electrically neutral, required number of Na+ and Cl− ions were added while 0.15 mol/L salt concentrations were set in all the systems. Using the steepest descent method, all the solvated systems were subjected to energy minimization using the steepest descent method for 5000 steps. Afterwards, NVT (constant number of particles, volume, and temperature) series, NPT (constant number of particles, pressure, and temperature) series, and the production run were conducted in the MD simulation [25]. The NVT and the NPT series were conducted at a 300 K temperature and 1 atm pressure for a duration of 300 ps. V-rescale thermostat and Parrinello-Rahman barostat were selected of the performed simulation. Finally, the production run was performed for a duration of 100 ns (nanoseconds) at 300 K. Thereafter, post-analysis was performed measuring root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA) and hydrogen bonds to analyze their stability. The Xmgrace [26] program was used to represent all the analyses in the form of plots.

2.4. Molecular dynamics (MD) simulation analysis

SwissADME [27] online tool (http://www.swissadme.ch) was utilized to evaluate the pharmacokinetics properties of the four compounds.

3. Results

3.1. Molecular Docking Analysis of phytochemicals with Dipeptidyl peptidase-4

AutoDock 4.2 predicted nine possible binding positions for each compound as output. The best position was chosen for each compound based on the lowest docking energy. The docking energy score of all the four compounds (figure 01) with DPP4 ranged from -6.8 to -7.1 Kcal/mol. The amino acid interactions of DPP4 with the compounds were also identified. Cytisine and N-methyl-cytisine showed docking energies of -7.1 and -6.8 Kcal/mol respectively with DPP4. Both compounds established 2 hydrogen bonding interactions with the protein while, Phe357 and Tyr547 were common interacting residues (figure 02). On the other hand, epilupipine and epilupipine-N-oxide showed docking energies of -7.0 and -6.9 Kcal/mol respectively with DPP4 with Glu205 and Glu206 being common interacting residues (figure 03). All the compounds established interactions with the key residues of the DPP4 protein indicating good stability among them.

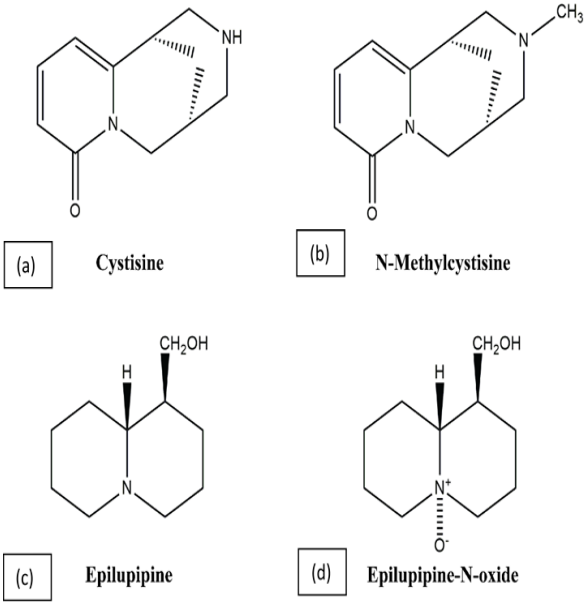

Figure 01: The four Alkaloids compounds cytisine (a), N-methyl-cytisine (b), Epilupipine (c), and Epilupipine-N-oxide (d).

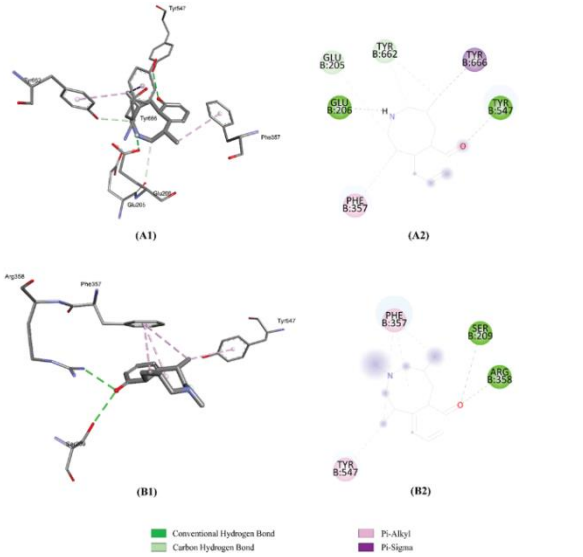

Figure 02: Interactions of cytisine (A1-A2) and N-methyl-cytisine (B1-B2) with DPP4.

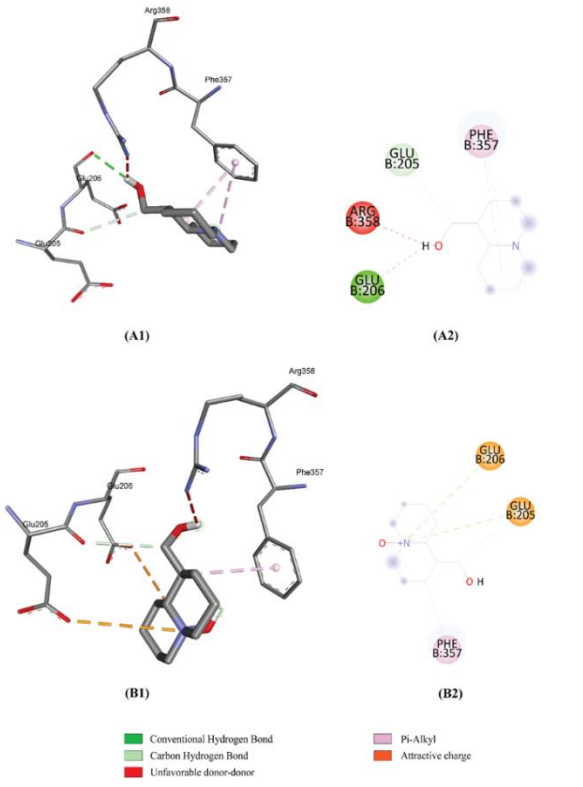

Figure 03: Interaction of epilupipine (A1-A2) and epilupipine-N-oxide (B1-B2) with DPP4.

3.2. *Molecular dynamics (MD) simulation results*

The dynamic movements of atoms and conformational variations of Cα atoms of the protein-ligand complexes were calculated by RMSD to detect their stability. It is observed that all the complexes exhibit lower RMSD (<0.4 nm) with no major fluctuations indicating their greater stability shown in figure 04. After some minor

fluctuations until 60ns, all the complexes retained stability till the end of the simulation period. The flexibility of each residue was calculated in terms of RMSF to get better insight on the region of proteins that are being fluctuated during the simulation. It can be understood that the binding of compounds makes the protein slightly flexible in 200-300 and 650-700 residue areas (figure 04). The compactness of the complex was represented by the radius of gyration (Rg). The lower degree of fluctuation throughout the simulation period indicates the greater compactness of a system. The Rg of the complexes was found to be lower than the beginning period while the DPP4-cytisine complex was the most compact (figure 05). Interaction between protein-ligand complexes and solvents was measured by solvent accessible surface area (SASA) over the simulation period. So, SASA of the complex was calculated to analyze the extent of the conformational changes happened during the interaction. Interestingly, the protein featured a slight reduction of the surface area showing a relatively lower SASA assessment than the initial period (figure 05). Hydrogen bonding between a protein-ligand complex is crucial to stabilize the structure. It was observed that the highest number of conformations of the protein formed up to two hydrogen bonds with the compounds while cytisine and N-methyl-cytisine formed the highest number of hydrogen bonds with DPP4 (figure 05).

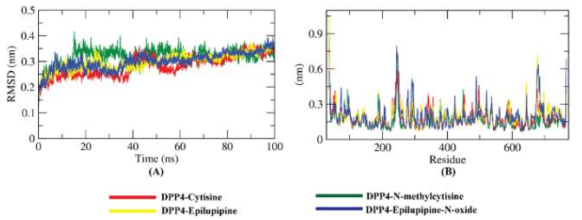

Figure 04: RMSD (A) and RMSF (B) results of the four protein-ligand complexes after 100ns simulation.

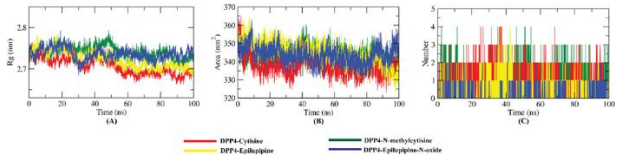

Figure 05: Rg (A), SASA (B) and Hydrogen bond (C) analysis results of the four protein-ligand complexes after 100ns simulation.

3.3. ADME analysis

The ADME properties of the four compounds were predicted using the Swiss ADME server (Table 01). In many attributes all the four compounds showed significant values. Interestingly, no compounds violate lipinski's rule of five and they have high GI absorption. The compounds do not inhibit any isoform of the cytochrome P450 enzyme. The log kp (skin permeation) was in good range and they also had efficacy in crossing the blood brain barrier. The PAINS alert confirmed the absence of catechol moieties in the compounds and synthetic accessibility values were also low in all the four compounds.

The compounds showed better results in both binding with the protein and in drug likeliness properties. Considering binding affinities, interactions with target proteins and ADMET profile, we have analyzed and validated the stability of protein-ligand complexes using MD simulation approach and found intriguing results for all of them. The results of this study might be valuable references for further experimental research in designing new potent DPP4 inhibitors.

4. Discussion

Should Computer aided drug design (CADD) approaches are currently used efficiency in the drug discovery and development course besides traditional methods. Among several approaches of CADD that are evaluated as favorable techniques, molecular docking and molecular dynamics simulations are mostly used to perform virtual screening of compounds to analyze how the ligands inhibit a target confirming their stability towards specific target proteins. Also, pharmacological properties of compounds can be assessed using the increasing development of robust computational tools before entering into experimental trial. So, intensive CADD methods were used in this study to assess drug gable properties of four compounds against DPP4 protein.

In this study, we focused on both the binding affinity and ADMET properties of the four compounds to test their efficacy against DPP4 which an intrinsic membrane glycoprotein is playing a major role in glucose metabolism and provides a useful treatment for diabetes mellitus type 2. For this, four compounds, cytisine, N methyl-cytisine, epilupipine and epilupipine-N-oxide were selected and docked against the DPP4.

Parameters	Cytisine	N-methyl-cytisine	Epilupipine	Epilupipine-N-oxide
Num. H-bond acceptors	2	2	2	2
Num. H-bond donors	1	0	1	2
Molecular weight (g/mol)	205.30	219.32	169.26	186.27
Lipinski violation	0	0	0	0
GI absorption	High	High	High	High
BBB permeant	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	No	No
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
Log Kp (skin permeation) (cm/s)	-6.35	-6.10	-6.45	-6.90
PAINS	No	No	No	No
Brenk	No	No	No	No
Synthetic accessibility		4.23	4.33	2.53

Table 01: Important ADME properties of the four compounds

It was found that cytisine exhibits the highest binding affinity of -7.1 kcal/mol while binding with the DPP4 and interacted with the highest number of amino acid residues in the active site. As we addressed earlier, despite having a high binding affinity of these compounds, we further tested their stability with DPP4 running MD simulation for 100 ns time period.

MD simulation results confirmed the stability of the four inhibitors with the DPP4. The RMSD plot revealed that all four compounds are stable and the RMSD values did not show any sudden fluctuation throughout the period. RMSF analysis confirmed that the complexes did not show much fluctuations and were in an acceptable range. Each complex had relatively similar behavior of compactness revealed from the radius of gyration (Rg) results. The SASA values depicted that the complexes neither increased nor decreased much in volume. A good number of hydrogen bonds was observed in four complexes throughout the simulation that explained their conformational stability once again. Thus, these compounds have the ability to bind with the DPP4 and interfere with its action.

The ADME properties of the four compounds describe high gastrointestinal absorption potency with a skin permeation coefficient (Log Kp) ranging from of −6.10 to -6.90. No compounds violate Lipinski's rule of five, thus, they might easily mimic with native ligand. Also, results of blood–brain barrier membrane permeability reveals that the compounds can cross the blood brain barrier. The Pan-assay interference compounds (PAINS) are chemical compounds that tends to give false positive results in high-throughput screens reacting nonspecifically with various biological targets. No compounds possessed any catechol group revealed from PAINS filter. All the four compounds have a low score of synthetic accessibility denoting that these compounds can be synthesized much more easily in experimental laboratories.

As the present study has been conducted through in-silico analysis, there might be some limitations here. The four compounds although they showed tremendous results with DPP4 protein, are yet to be performed in an animal model experiment. So, adequate experimental validations are needed to ensure their therapeutic efficacy.

5. Conclusions

In conclusion potentiality of four compounds targeting dipeptidyl peptidase-4 (DPP4), a type II transmembrane glycoprotein that plays vital role in metabolism of glucose . DPP-4 inhibitors reduce glucagon and blood glucose levels. The mechanism of DPP-4 inhibitors is to increase incretin levels (GLP-1 and GIP),which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels. We performed molecular docking analysis, tested the stability of the complexes using molecular dynamics simulations and explored their pharmacokinetic properties. The binding energy of the compounds raged from -6.8 to -7.1 Kcal/mol. Further, the post molecular dynamics (MD) analysis show that all of them were greatly stable with DPP4 having similar results. Pharmacokinetic parameters of the compounds revealed very good properties in terms of adsorption, distribution, metabolism and excretion.

Our study showed that these four compounds may turn out to be potent in treating malfunctioning of DPP4 to maintain glucose levels. They are yet to be performed in animal model experiment. So, adequate experimental validations are needed to ensure their therapeutic efficacy.

6. Conflicts of interest

"There are no conflicts to declare".

7. Formatting of funding sources

I am funding myself

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