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Veratric Acid as a Potential Antidiabetic Agent: Molecular Docking and *In vivo* Enzyme Inhibition Studies with Insights from STz-NA Induced Diabetic Rat Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

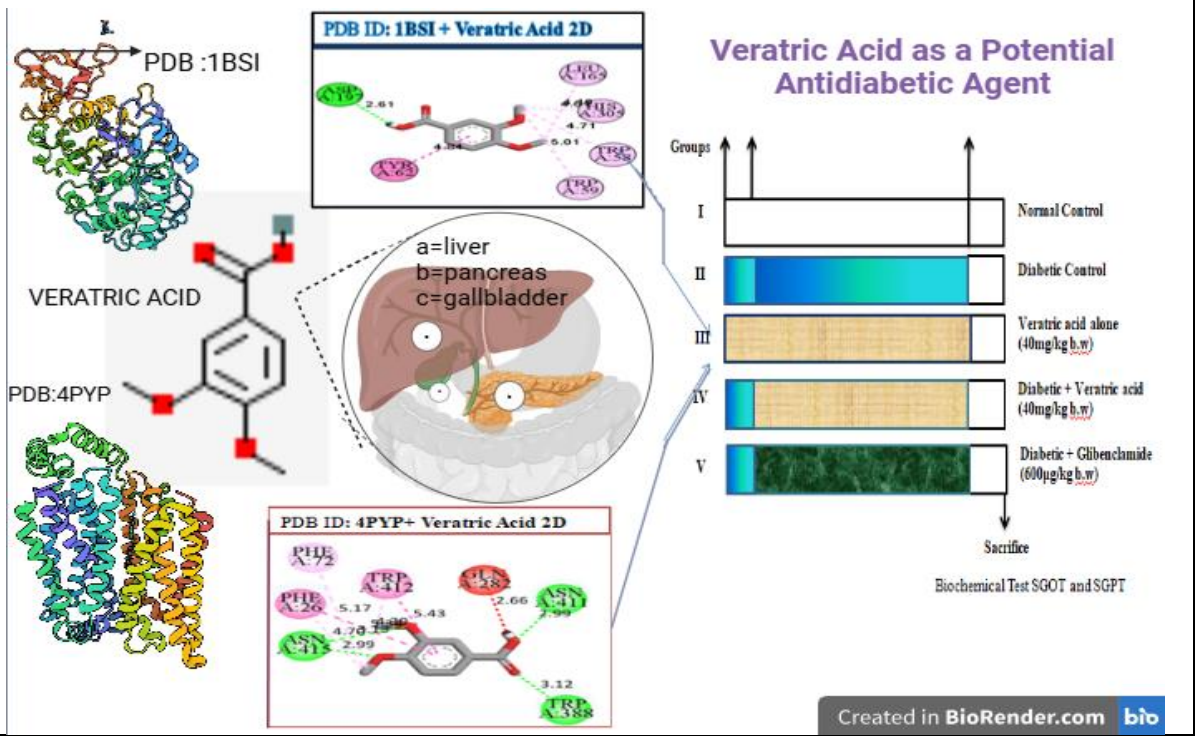
Streptozotocin-nicotinamide (STz-NA) has proven to be an effective agent in inducing type 2 diabetes (T2DM) in experimental models due to its genotoxic impact on pancreatic β-cells. STz reduces nicotinamide adenine dinucleotide (NAD^+) through the glucose transporter GLUT2, leading to cellular damage, DNA strand breaks, and cell death. Nicotinamide (NA), a biochemical precursor of NAD^+ and poly-ADP-ribose-polymerase-1 (PARP-1) inhibitor, plays a crucial protective role by modulating NAD^+ levels, which are essential in ATP production and other metabolic processes. Excessive DNA damage, however, triggers PARP-1 over-activation, depleting cellular reserves and resulting in cell necrosis.

This study further investigates the antidiabetic and hepatoprotective effects of Veratric Acid (VA) by comparing it to Glibenclamide. Utilizing Computer-Aided Drug Design (CADD), VA was identified as a structurally similar compound with potential antidiabetic properties. Molecular docking studies targeting proteins, such as GLUT-1 (PDB ID: 4PYP) and 1BSI, along with in vivo assays in STz-NA-induced diabetic Wistar rats, were conducted. VA demonstrated significant binding affinity and molecular interactions comparable to standard antidiabetic agents, showing inhibitory effects on liver enzymes SGOT and SGPT, and supporting its potential role in diabetes management.

Docking and histopathology results revealed that VA effectively reduced blood glucose, improved insulin levels, and enhanced liver function in diabetic rats. Additionally, VA promoted insulin secretion from pancreatic β-cells and upregulated insulin-related markers, with minimal hepatotoxicity compared to Glibenclamide. VA treatment significantly improved enzymatic and non-enzymatic antioxidant levels, lowered lipid peroxidation, and improved lipid profiles, which were confirmed by histopathological liver observations.

Veratric Acid at low doses effectively modulates blood glucose and diabetes-related biochemical parameters, highlighting its promise as a safer, natural therapeutic alternative for diabetes treatment.

GRAPHICAL ABSTRACT



Keywords: Veratric acid; glibenclamide; CADD; molecular docking; antidiabetic properties; Streptozotocin (STz); Nicotinamide (NA); GLUT-1; pancreatic β-cells; hepatotoxicity.

1. INTRODUCTION

Worldwide, diabetes is a major public health challenge, affecting hundreds of millions and contributing to significant morbidity and mortality. It is estimated that around 240 million individuals currently live with undiagnosed diabetes, while nearly half of all adults with the disease remain unaware of their condition. The burden of diabetes continues to grow rapidly. In 2021, the International Diabetes Federation (IDF) reported that 537 million people were living with diabetes, which corresponds to approximately 10.5% of the global adult population. This figure is expected to escalate sharply; projections indicate a rise to 643 million cases (11.3%) by 2030 and 783 million cases (12.2%) by 2045.

India, in particular, faces a significant diabetes crisis. With the second highest number of diabetic patients globally, India had an estimated 74.9 million individuals aged 20–79 living with diabetes in 2021. Alarmingly, this figure is projected to surge to 124.9 million by 2045, largely driven by urbanization, lifestyle changes, and an aging population. The economic and healthcare implications are substantial, as diabetes management and complications place a heavy burden on both individuals and the healthcare system. Addressing the diabetes epidemic requires urgent global and national strategies focused on prevention, early detection, and effective management to curb this growing health crisis (Hossain et al., 2024, Maiti et al., 2023).

1a. Streptozotocin, chemically known as 2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose [Fig. 1], is an antibiotic derived from the bacterium Streptomyces achromogenes. Its structure and mechanism give it a unique ability to selectively target pancreatic β-cells due to its high affinity for β-cell membrane transporters. This selective toxicity makes streptozotocin widely used in research for inducing diabetes in animal models, as it effectively destroys β-cells and thereby simulates diabetic conditions (Raju et al., 2011). However, the impact of streptozotocin extends beyond the pancreas. Its cytotoxic properties can also inflict damage on other organs, notably the kidneys, liver, and intestines, leading to potential complications in experimental settings (Deeds et al., 2011).

A key outcome of β-cell destruction by streptozotocin is chronic hyperglycemia, a condition characterized by persistently elevated blood glucose levels. Chronic hyperglycemia is commonly associated with diabetes, where it serves as a defining feature. However, this condition can also arise from other health disorders or lifestyle factors, such as metabolic syndrome, prolonged stress, or dietary habits that maintain high blood sugar. Prolonged hyperglycemia can lead to serious complications, affecting multiple organ systems, including the cardiovascular system, kidneys, eyes, and nerves. This makes understanding and managing chronic hyperglycemia crucial not only for diabetes patients but also for those with other risk factors that may contribute to elevated blood glucose. A complex interplay between nutrition and lifestyle leads to the etiology of type 2 diabetes mellitus (Galicia-Garcia et al., 2020).

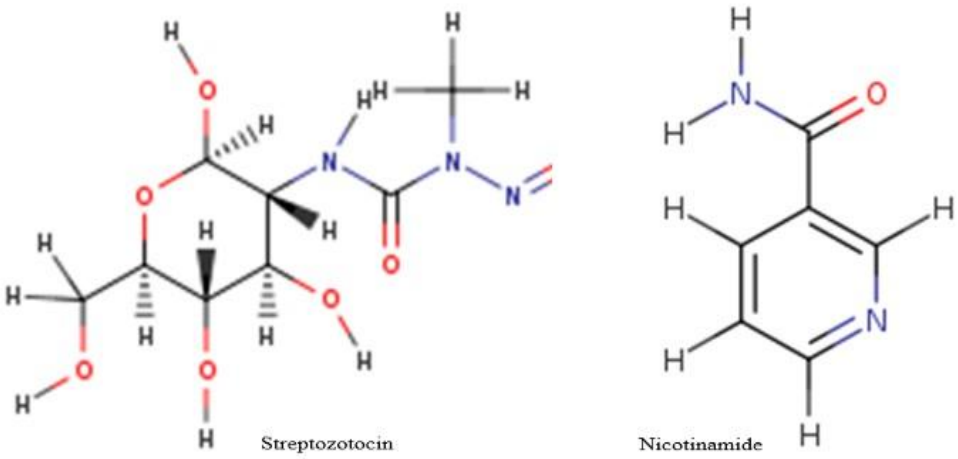


Fig.1. Molecular structure of Streptozotocin($C_8H_{15}N_3O_7$) and Nicotinamide $C_6H_6N_2O$

Dysregulation of lipid patterns is a major consequence of diabetes mellitus. Diabetes, particularly type 2, is often accompanied by characteristic changes in lipid profiles, known as diabetic dyslipidemia. This includes well-documented alterations such as increased levels of triglycerides, cholesterol, and LDL cholesterol, along with decreased levels of HDL cholesterol (Sophia and Manoharan, 2007,Pamu et al., 2017). Lipid profile alteration increases the risk of cardiovascular disease in diabetic patterns. The relationship between lipids and diabetes mellitus is a complex phenomenon. Dysregulated lipid metabolism prone into the development and complications of diabetes (Mooradian, 2009).

1.b. Nicotinamide (Niacinamide) [Fig.1]plays a key role in energy production and cellular metabolism.Nicotinamide is a precursor of nicotinamide adenine dinucleotide (NAD^+), a vital coenzyme involved in redox reactions in cells. NAD^+ plays a crucial role in cellular metabolism by participating in energy production, DNA repair, and cell signaling processes. By supporting these pathways, nicotinamide helps in maintaining healthy cellular function, energy balance, and tissue repair.Its role in energy metabolism and cellular repair makes it essential for overall health, and it is generally well-tolerated with minimal side effects at standard dosages.

1.c. Veratric acid [Fig. 2] is a natural phenolic compound primarily found in the stem of Tabebuia impetiginosa and in the medicinal mushroom Sparassiscrispa. It is also present in smaller concentrations in various fruits and vegetables. Known for its significant pharmacological properties, veratric acid has been widely studied for its potential health benefits, particularly in combating oxidative stress, hyperlipidemia, and inflammation.

Research by Yu et al.,(2021) demonstrated that veratric acid activates the Nrf2 signaling pathway, a key regulator in cellular defense against toxic and oxidative mechanisms, which protects the liver from oxidative damage and drug detoxification. In other studies, Saravanakumar and Raja,(2011) observed that veratric acid exhibits both antioxidant and anti-hyperlipidemic effects in hypertensive rat models. Likewise, Raja et al.,(2012) reported similar antioxidant and lipid-lowering properties in Wistar rats fed an atherogenic diet, indicating the potential of veratric acid to improve cardiovascular health by reducing oxidative stress and cholesterol levels.

Beyond cardiovascular and antioxidant benefits, veratric acid has also shown promise in dermatological and immunological applications. Lee et al., (2016) found that veratric acid helps prevent UV-induced premature skin aging, underscoring its potential in skincare formulations. Additionally, Choi et al.,(2015,2012) demonstrated that veratric acid reduces iNOS production in lipopolysaccharide (LPS)-stimulated macrophage cells through histone acetylation and PI3K pathway activation. This regulatory effect extends to nitric oxide generation, suggesting that veratric acid can modulate inflammatory responses, a property valuable in managing inflammatory diseases.

Further studies, such as those conducted by Ran et al.,(2014), indicate that veratric acid is effective in protecting against acute lung injury in LPS-challenged mice, likely due to its ability to inhibit NF-κB expression and thereby attenuate inflammatory injury. Palko-Labuz et al. (2021) explored an innovative approach by conjugating veratric acid with phospholipids, significantly enhancing its anticancer efficacy against melanoma cells. Additionally, its free radical scavenging abilities have been assessed *In vitro*, notably in KB cells using specialized colorimetric assays (Sivasankaran, 2023).

The present study investigates the antidiabetic and antihyperlipidemic effects of veratric acid in streptozotocin (STz)-induced diabetic rats, aiming to expand upon its known pharmacological actions and explore its potential as a therapeutic agent for diabetes and related metabolic disorders (Al-Joufi, 2020).

1.d. Glibenclamide (Glyburide) [Fig. 2] is a commonly used drug from the sulfonylurea class. It helps control blood sugar levels by stimulating the pancreas to release insulin (Lv, 2020). The drug works by binding to sulfonylurea receptors (SUR1) on pancreatic β-cells, which closes ATP-sensitive potassium channels (Seino, 2012). This action depolarizes the cell membrane, opens calcium channels, and increases intracellular calcium levels, triggering insulin secretion (Velasco, 2016). It is prescribed to manage blood glucose in patients with type 2 diabetes (Blonde et al., 2017). when diet and exercise are insufficient, and is often part of combination therapy with other antidiabetic agents like metformin, insulin, or glipizide (Mudaliar and Henry, 1999).

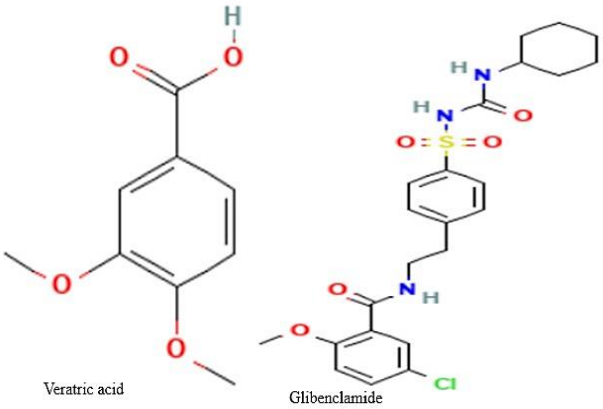


Fig.2. Molecular structure of Veratric acid ($C_9H_{10}O_4$)and Glibenclamide ($C_{23}H_{28}ClN_3O_5S$)

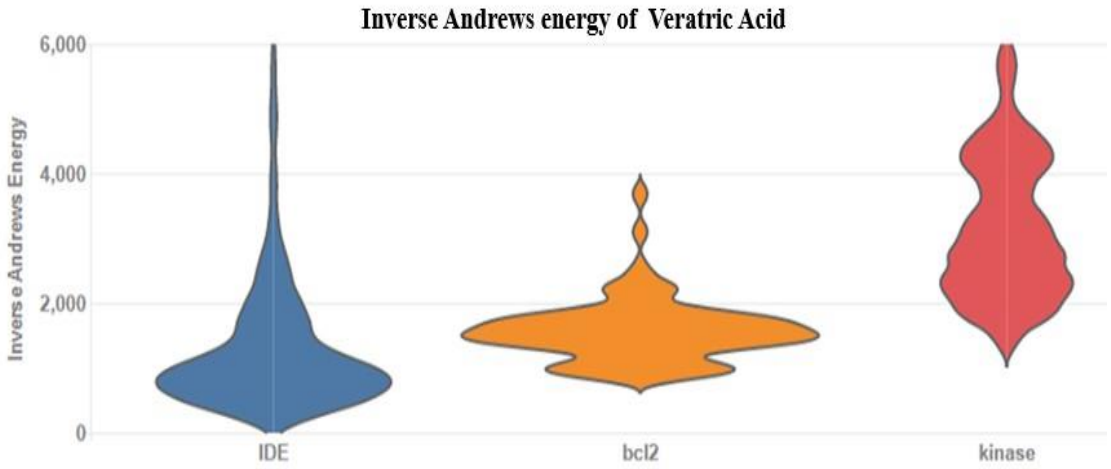


Fig.3. Inverse Andrews energy of Veratric Acid

Glibenclamide vs. Metformin: While glibenclamide stimulates insulin release, metformin primarily reduces glucose production in the liver and enhances insulin sensitivity, usually without causing hypoglycemia (Phielix et al., 2011).

Glibenclamide vs. Insulin: Unlike insulin, glibenclamide is taken orally and works by promoting the release of insulin, rather than replacing it directly (Frederico et al., 2017).

Glibenclamide vs. Glipizide: Both drugs belong to the sulfonylurea class, but glipizide has a shorter half-life and may pose a lower risk of prolonged hypoglycemia (Rendell, 2004).

The image [Fig.3] displays violin plots that compare the "Hydrophobic Ratio," "Hydrophobic or Polar Ratio," and "Mean Axis" across three protein targets: IDE (Insulin-Degrading Enzyme), BCl_2, and Kinase.

Insulin-degrading enzyme (IDE) plays a crucial role in insulin regulation, and its structural characteristics provide insight into its functionality (Hulse, 2009). IDE exhibits a wide variation in its hydrophobic ratio, indicating diverse surface hydrophobicity that may impact ligand binding and enzymatic degradation (Stefanidis et al., 2018). Additionally, IDE's relatively high hydrophobic-to-polar ratio compared to proteins like BCl_2 and kinase suggests a more hydrophobic surface, which enhances its ability to interact with hydrophobic regions of insulin. Furthermore, IDE's mean axis value is broader thanBCl_2's and closer to kinase, implying greater structural flexibility that allows it to bind larger substrates like insulin more effectively(Waraky, 2017). These structural insights highlight IDE's

adaptability and efficiency in interacting with insulin, making it a key target for further research in insulin regulation and potential therapeutic interventions (Tundo et al., 2023).

1.e. Ligand ability of IDE: Insulin-degrading enzyme (IDE), also known as IDE HUMAN, is a challenging target for drug discovery due to its high hydrophobic ratio and structural complexity, which complicates the identification of small-molecule inhibitors. However, certain regions may be more accessible to hydrophobic ligands, as supported by violin plot analysis highlighting the importance of hydrophobic interactions in its ligand-binding potential(Raman and MacKerell, 2015). IDE plays a critical role in the breakdown of various peptides, including insulin, APP peptides, IAPP peptides, natriuretic peptides, glucagon, and bradykinin, contributing to intercellular peptide signaling (Tundo et al., 2017). Substrate binding induces significant conformational changes, enabling the degradation of larger molecules like insulin (Shen et al., 2006). This function is essential for regulating peptide hormone signaling cascades and maintaining blood glucose homeostasis. Additionally, IDE is involved in the degradation and clearance of amyloidogenic peptides linked to Alzheimer's disease, as well as natriuretic peptides that regulate intracellular cGMP levels. IDE also degrades an aberrant 40-residue form of NPPA (fsNPPA), which is associated with familial atrial fibrillation in certain patients (Frisardi et al., 2010).

The distribution analysis of Inverse Andrews Energy across different molecular targets, including IDE (Insulin-Degrading Enzyme), BCl_2 (B-cell lymphoma 2), and kinase, offers important insights into the potential binding interactions and stability of Veratric Acid within these systems.

The analysis of Inverse Andrews Energy distribution reveals diverse binding properties of Veratric Acid across three molecular targets: IDE, BCl_2, and kinase. For IDE, higher energy values suggest potential challenges for Veratric Acid to bind optimally, which may affect its therapeutic efficacy in managing insulin levels in diabetic conditions (Hao, 2018). In contrast, Veratric Acid shows more stable interactions with BCl_2, indicating its potential as a modulator of apoptosis, with implications for cancer therapies targeting BCl_2 overexpression (Kaur et al., 2018). The distribution for kinases suggests a specific affinity that could influence cellular signaling, offering potential therapeutic roles in metabolic diseases and cancer (Shchemelinin et al., 2006). Overall, these findings underscore the need for further research into Veratric Acid's mechanisms of action and therapeutic applications in diabetes and cancer management (Mujica et al., 2017).

Type 2 diabetes is closely linked to key molecular targets such as pancreatic alpha-amylase (PBD: 1BSI) [Fig. 4] and the glucose transporter GLUT1 (PBD: 4PYP)[Fig. 4]. Pancreatic alpha-amylase plays a significant role in carbohydrate metabolism by breaking down complex sugars into simpler glucose units, contributing to postprandial hyperglycemia in diabetic patients (Kashtoh and Baek, 2023). Inhibiting this enzyme can help reduce glucose spikes after meals (Kerimi et al., 2019), making it a target for diabetes management. On the other hand (Carbó and Rodríguez, 2023), GLUT1 is a critical glucose transporter involved in basal glucose uptake across cell membranes. Dysregulation of GLUT1 in type 2 diabetes can impair glucose transport, leading to elevated blood glucose levels. Targeting these proteins through therapeutic interventions can help regulate blood sugar levels and provide better control over type 2 diabetes.

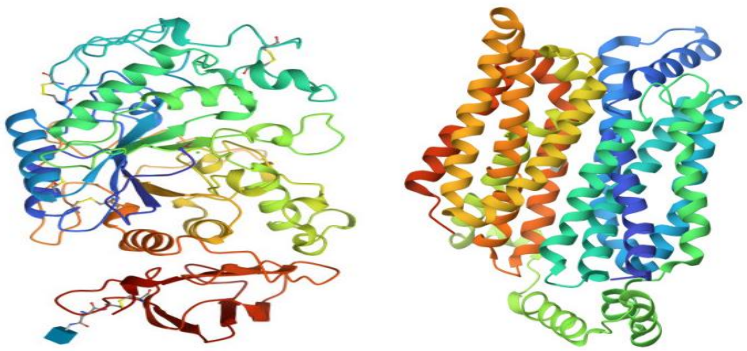


Fig.4. PBD:1BSI (Human Pancreatic alpha amylase); PBD: 4PYP(human glucose transporter GLUT1)

2. MATERIALS AND METHODS

2.a Chemicals: Veratric Acid, Streptozotocin, and Nicotinamide (NA) were obtained from Sigma Chemical Company (St. Louis, MO, USA). The rest of the chemicals were obtained from Hi Media Mumbai and were of analytical grade by calculation of pharmacokinetic parameters.

2.b.Calculation of pharmacokinetic parameters: In order to further optimize the molecules, all the phytoconstituents were tested for violating Lipinski's rule of five along with their binding affinity with the alpha- amylase enzyme (Sharma et al., 2021). The properties of all the phytoconstituents were calculated from SwissADME online tool (http://www.swissadme.ch/index.php) (Sokkar et al., 2020).

2.c.Molecular docking studies: The molecular docking (MD):The structures of all the phytoconstituents, synthetic drugs and native ligand (.sdf File format) were downloaded from the National Center for Biotechnology Information PubChem (https://pubchem.ncbi.nlm.nih.gov/). The energy minimization (optimization) was performed by Universal Force Field (UFF) (Drwal et al., 2014). Autodockvina 1.1.2 in PyRx 0.8 was used to perform the MD studies of all the phytoconstituents and native ligand against the crystal structure of alpha amylase enzyme. The enzyme structures, with the aid of Discovery Studio Visualizer 2024, were optimized, purified and prepared for MD. Molecules (PDB, PDBQT Files), both ligands as well as targets (Human Pancreatic alpha amylase) were selected for MD(Chaudhari et al., 2020). For the purpose of MD simulation, the three-dimensional grid box (size_x = 63.6943A0; size_y = 63.4700A0; size_z = 62.1868A0) was built using Autodock tool 1.5.6 with exhaustiveness value of 8(Dallakyan and Olson, 2015). The active amino acids in the protein were analyzed and illuminated using BIOVIA Discovery Studio Visualizer 2024 (version-19.1.0.18287)(San Diego: Accelrys Software Inc., 2012). The full MD process, the identification of cavity and active amino acid residues were performed as per the procedure described by S. L. Khan et al(Chaudhari et al., 2020; Khan, Sharuk L; Siddiui, 2020; Khan et al., 2022, 2020; S. Khan et al., 2021; S. L. Khan et al., 2021; Shntaif et al., 2021; Siddiqui et al., 2021)[Fig.5].

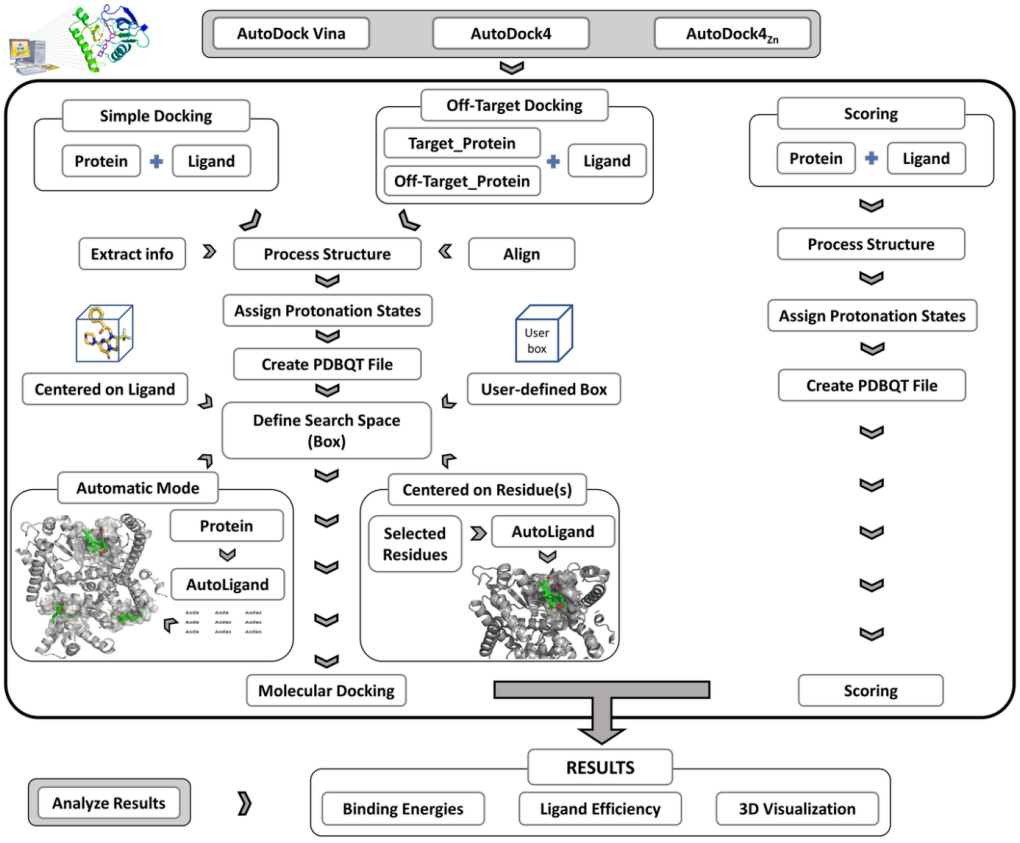


Fig.5. Structure of the compound molecules and ligand interaction methods flowchat

Table 1. The rats will be divided into five groups, with six rats per group

Group 1	Control rats
Group 2	Diabetic control ratsSTz[45mg/ kg body weight]+NA[110mg/ kg body weight]
Group 3	Normal+Veratric Acid (40mg/kg body weight)
Group 4	Diabetic rats treated with veratric acid (40mg/kg body weight)
Group 5	Diabetic rats treated with glibenclamide (0.6mg/kg body weight)

2.d.Experimental Animal model: Albino male Wistar rats (Rattus norvegicus, 180–200 g) were obtained from the Central Animal House of Rajah Muthiah Medical College and Hospital, Chidambaram. The rats were housed in plastic cages in a well-ventilated room with a 12-hour light/dark cycle. They were given free access to a standard pellet diet (from the National Institute of Nutrition Ltd, Bangalore, India) and water. This study was approved by the Institutional Animal Ethics Committee (IAEC) of Rajah Muthiah Medical College (IAEC Proposal No. Annamalai University-IAEC-1353/2/23) and was conducted according to the Guide for the Care and Use of Laboratory Animals.

2.e. Experimental Design: Group 1 rats served as control animals, Group 2 animals were treated with single dose on 30th day streptozotocin and after 15 minutes received nicotinamiteserved as diabetic control animals, Group 3 and Group 4 animals were treated with streptozotocin(STz) followed by the oral administration of veratric acid(VA)and standard drug glibenclamide respectively for 30 days. Group 5 rats were treated with veratricacidabove for a period of 30 days. After final dose animals are sacrificing and have taken blood for SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase)and body weight of initial and final.

3. RESULTS AND DISCUSSION

3.1 Heat Map-Colour Variance

The heat map image is displaying values of a primary variable across two axis variables with a color-coded grid for easy visual interpretation. Each square's color represents the value of the primary variable in that range, offering a color-coded, easy-to-interpret view of data density[Fig. 6].

3.1.a. Biochemical analyses: Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter (a-e) differ significantly at $p<0.05$ ANOVA [Duncan Multiple Range Test (DMRT)].

Discussion: According to the heat map and graph, it is clear that body weight decreased in Group 2 rats, compared to Group 1 weight, with values of 156.22 versus 211.81.Additionally, the heat map and graph show that Group 3 rats, exhibited a decrease in body weight compared to Group 4rats with values of 215.14g versus 205.31g.Furthermore, the data highlight that body weight decreased more in Group 3 rats compared to Groups 5, values were 215.14 g, and 201.51 g. respectively [Table2 and Graph-1].

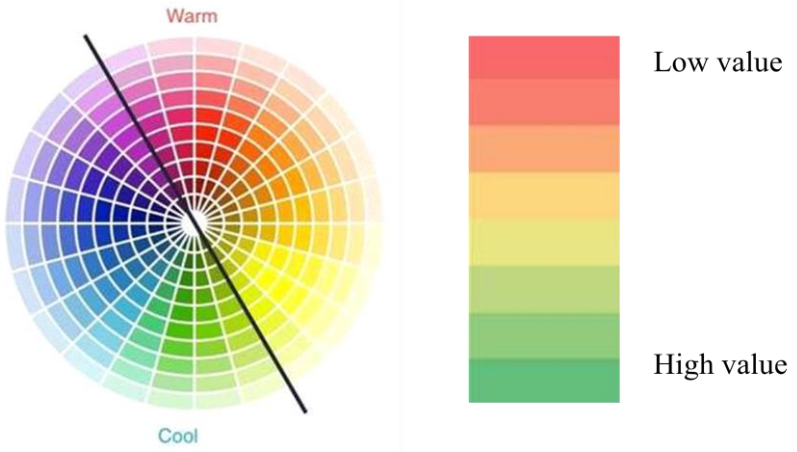
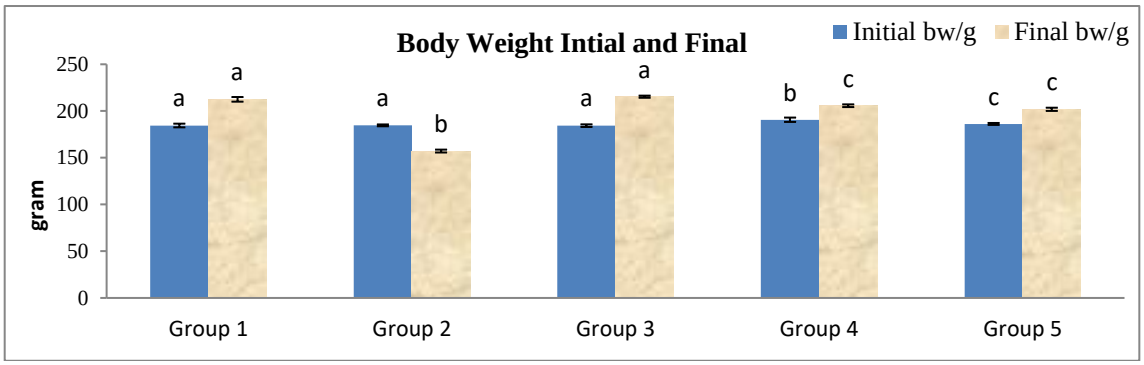


Fig.6. Heat map-colour variance

Table2. Body weight in STz diabetic rats before and after oral administration of VA

Groups		Body Weight (g)	
		Initial	Final
Group 1	Normal Control	184.50	212.50
Group 2	Diabetic Control [STz(45mg/ kg body weight)+NA(110mg/ kg body weight)]	184.67	147.22
Group 3	Normal+Veratric Acid (40mg/kg body weight)	184.33	215.33
Group 4	Diabetic + Veratric Acid (40mg/kg body weight)	190.67	205.67
Group 5	Diabetic+Glibenclamide (0.6 mg/kg body weight)	186.17	201.83

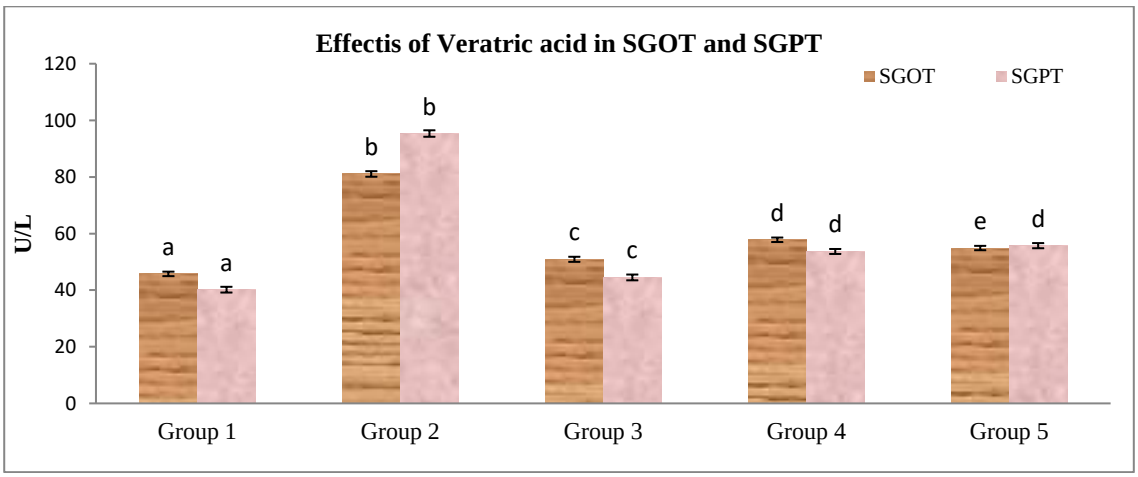


Graph 1. Body weight of rats. Each column is mean ± S.D. for six rats in each group. In each column, means with different letter (a–c) differ significantly at p=<0.05 ANOVA [Duncan Multiple Range Test (DMRT)]

Table 3. SGOT & SGPT in STz diabetic rats before and after oral administration of VA

Group		SGOT (U/L)	SGPT (U/L)
Group 1	Normal control	45.75	40.17
Group 2	Diabetic Control:STz (45mg/kg+15mint NA(110 mg/kg)b.w.	81.05	95.33
Group 3	Normal+Veratric acid (40mg/kg b.w)	50.90	44.50
Group 4	Diabetic+Veratric acid (40mg/kg b.w)	57.80	53.67
Group 5	Diabetic+Glibenclamide (0.6mg/kg b.w)	54.88	55.72

Values are given as mean ± S.D from six rats in each goup. Values not sharing a common superscript letter (a-e) differ significantly at p<0.000 and p<0.005 ANOVA (DMRT).



Graph 2. SGOT & SGPT test of rats. Each column is mean ± S.D. for six rats in each group. In each column, means with different letter (a–c) differ significantly at p=<0.05 ANOVA [Duncan Multiple Range Test (DMRT)]

Discussion: The effects of veratric acid and glibenclamide on liver enzymes, SGPT and SGOT were studied in both normal and diabetic rats. Elevated levels of these enzymes are indicators of liver damage. According to the heat map and graph, baseline enzyme levels in Group 1 (control) were SGOT: 45 and SGPT: 40. In Group 2, which consisted of rats with streptozotocin-induced diabetes, there was a significant increase in SGOT: 81 and SGPT: 95, reflecting liver damage In Group 3, treated with veratric acid under normal conditions, a slight increase in SGOT: 51 and SGPT: 45 was observed, indicating mild liver stress. However, in the diabetic control Group 4, veratric acid treatment reduced SGOT to 58 and SGPT to 53, suggesting a protective effect on the liver. Similarly, Group 5, which received glibenclamide treatment, showed SGOT: 55 and SGPT: 55, levels close to those in the veratric acid-treated group, indicating that glibenclamide also contributed to reducing liver enzyme levels in diabetic rats [Table3 and Graph2].

3.1.b. Docking Studies analysis:

Discussion: Docking studies will based on the Lipinski rule of 5 and Veber's rule[Table 4].Medicinal chemists and pharmacologists have long sought drug-like chemical properties that reliably lead to orally effective therapeutic agents. Lipinski's "rule of five" is a key experimental and computational guideline used to predict a compound's solubility, membrane permeability, and overall efficacy during drug development. According to this rule, an orally active drug is more likely if:

- The compound has no more than 5 hydrogen bond donors (e.g., OH or NH groups).
- The compound has no more than 10 hydrogen bond acceptors (e.g., N or O atoms).
- The molecular weight is less than 500 Daltons.
- The octanol-water partition coefficient (log P) is less than 5, indicating appropriate lipophilicity.

These criteria help estimate solubility and permeability, two key factors in drug absorption. The rule is named because each criterion is based on multiples of five. It states that an orally active drug should meet these conditions, with no more than one exception, to be considered likely to succeed in terms of absorption and efficacy.

GenerallyVeber's rule is used in molecular docking to asses of bioavailability of compounds in orally when conjucate with Lipinski's five rule. In this rule is based on rotatable bonds and polar surface area of compounds. According the rule

(i) The number of rotable bonds (RB) in a compound should be less than 10. And
(ii) The polar surface area (PSA) of a compound should be no greater than 140. The number of rotatable bonds is important for measuring a drug.

The pharmacokinetics and drug-likeness properties of phytoconstituents are tabulated in Table 5. The physicochemical radar of the molecules in which the colored zone is the suitable physicochemical space for oral bioavailability is illustrated in Fig. 5. The ligand energies (kcal/mol), binding free energy (kcal/mol), and the active amino residues, bond length (A0), and type of interactions of phytoconstituents with alpha amylase enzyme are depicted in Table 3. The 2D- and 3D-docking poses of all the docked molecules are represented in Table 5.

Pharmacokinetic and drug-likeness properties significantly influence the therapeutic potential of compounds. Streptozotocin, for instance, demonstrates low gastrointestinal (GI) absorption and cannot cross the blood-brain barrier (BBB), limiting its oral bioavailability and central nervous system (CNS) application. Its status as a P-glycoprotein (P-gp) substrate further reduces its effectiveness by promoting its expulsion from cells. Nicotinamide, on the other hand, shows high GI absorption but also lacks BBB permeability, although it is not a P-gp substrate, potentially improving its systemic availability for peripheral applications. Veratric Acid stands out with high GI absorption, the ability to cross the BBB, and a favorable bioavailability score, making it an attractive candidate for systemic and neuroprotective therapies. Glibenclamide, like Streptozotocin, has low GI absorption and cannot penetrate the BBB, although it is not a P-gp substrate, limiting its systemic therapeutic potential. In comparison, Veratric Acid emerges as the superior compound due to its comprehensive pharmacokinetic advantages, making it promising for conditions requiring both systemic and CNS effects, such as diabetes management and neuroprotection. Conversely, the limitations in absorption and BBB permeability of Streptozotocin, Nicotinamide, and Glibenclamide highlight challenges that must be

addressed for their therapeutic application. Veratric Acid stands out for its promising pharmacokinetic properties, which could enhance its clinical efficacy in the treatment of metabolic disorders.

The image [Fig. 7] depicts a "boiled egg" plot from SwissADME, which predicts the gastrointestinal absorption (HIA) and brain penetration (BBB) potential of small molecules. Four molecules are represented: Veratric acid, Glibenclamide, Nicotinamide, and Streptozotocin. Veratric acid (Molecule 1) is located in the yellow region, indicating high gastrointestinal absorption but no brain penetration. Glibenclamide(Molecule 2) is near the edge of the plot, suggesting low brain penetration potential and possibly being a P-glycoprotein (PGP) substrate. Nicotinamide (Molecule 3) is closer to the white area, reflecting moderate absorption potential. Streptozotocin (Molecule 4) is in the bottom right, indicating low absorption and limited brain penetration. The plot's background is divided into two zones: the yellow area indicates high GI absorption but no BBB permeability, while the white area suggests both GI absorption and BBB penetration. Legends classify molecules based on BBB permeability, HIA and their status as P-gp substrates.

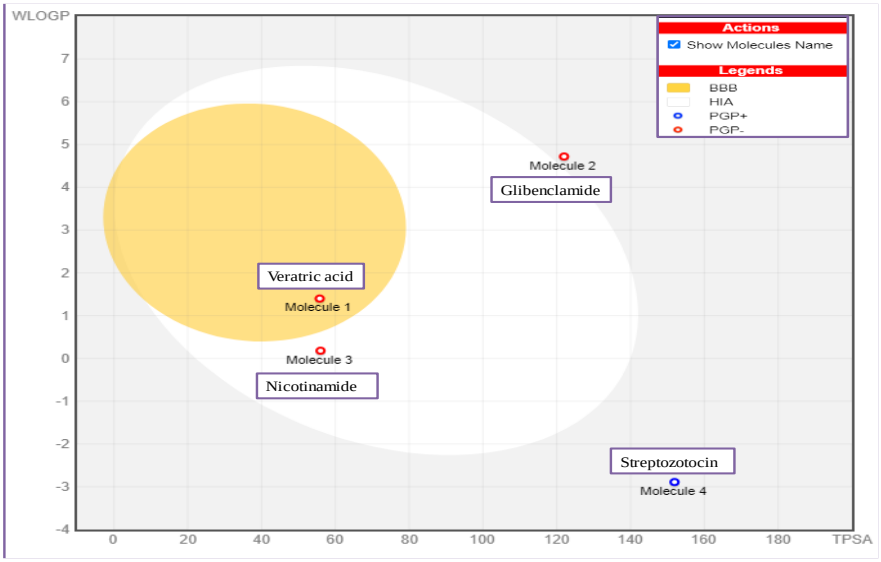


Fig.7. A"boiled egg" plot from SwissADME prediction of gastrointestinal absorption and brain penetration of small molecules in docking studies

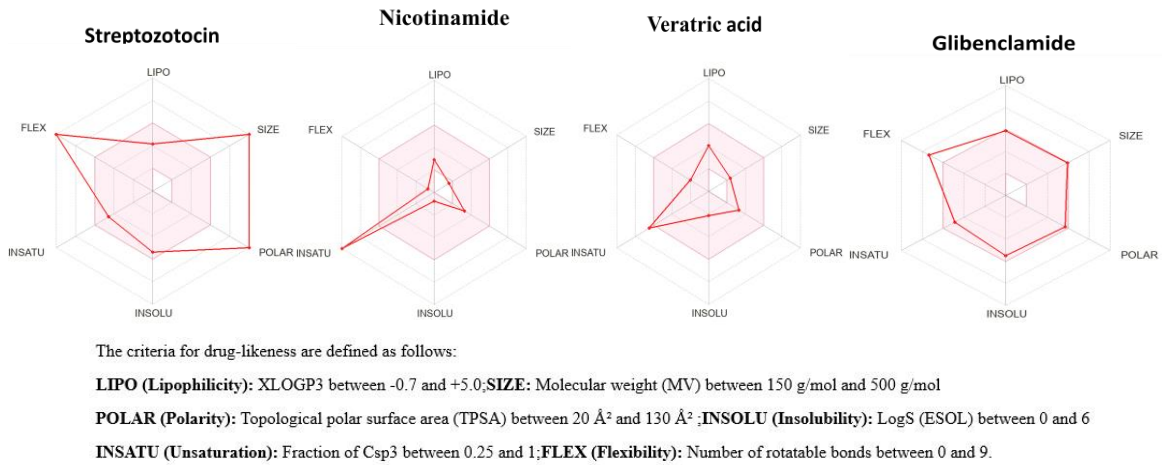


Fig.8. The physicochemical radar of the molecules in which the colored zone is the suitable physicochemical space for oralbioavailability

Table 4. The Lipinski rule of 5 and Veber's rule of the docked phytoconstituents

Compound Names	Lipinski Rules of 5						Veber's rule	
	Mass g/mol	Hydrogen Bond Donor	Hydrogen Bond Acceptors	logP	Molar Refractivity	Violation	Total polar surfacce area (Å)	No. of rotatabble bonds
Streptozotocin	265	5	10	-2.890901	55.856892	0	151.92	5
Nictotinamide	122	2	1	0.36717	26.703299	0	55.98	1
Veratric acid	181	0	4	0.0673	43.876495	0	55.76	3
Glibenclamide	473	3	8	4.377519	125.98056	0	121.98	11

Table 5. The pharmacokinetics and drug-likeness properties of phytoconstituents:

MoleculesNames	Pharmacokinetics									Drug-likeness			
	GI absorbtion	BBB permeant	P-gp substrate	CYP1 A2	CYP2 C19	CYP2 C9	CYP2 D6	CYP3 A4	Log Kp (skin permeation, cm/s)	Ghose	Egan	Muegge	Bioavai lability Score
Streptozotocin	L	N	Y	N	N	N	N	N	-8.55	N	N	N	0.55
Nicotinamide	H	N	N	N	N	N	N	N	-7.31	N	Y	N	0.55
Veratric acid	H	Y	N	N	N	N	N	N	-6.27	Y	Y	N	0.85
Glibenclaimde	L	N	N	N	Y	Y	Y	Y	-5.90	N	Y	Y	0.55

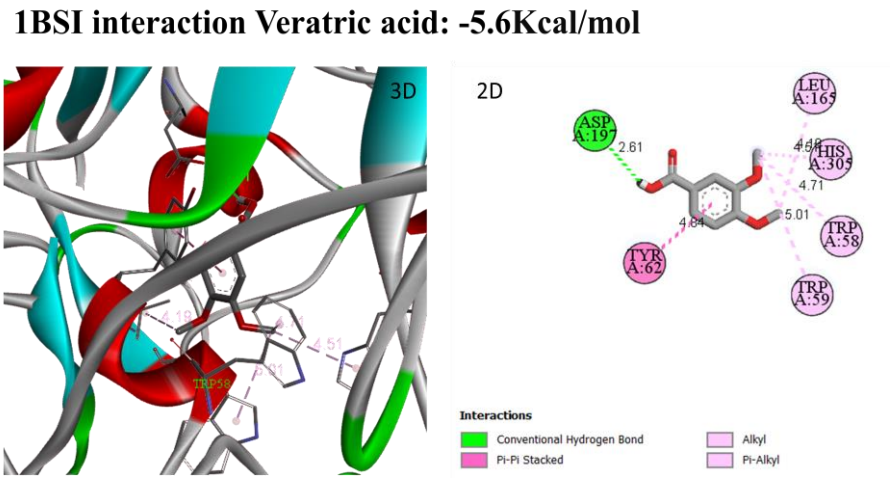


Fig.9. PDB:1BSI interaction with Veratric acid 3D and 2D view

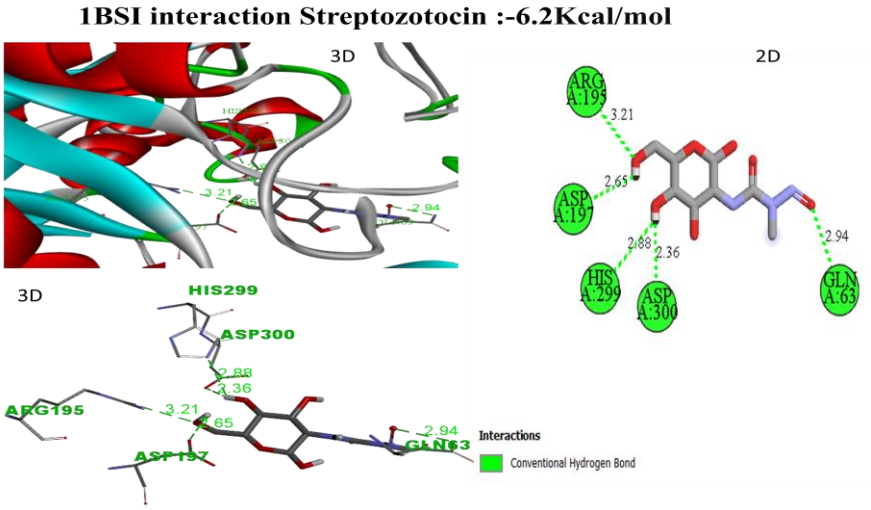


Fig. 10. PDB:1BSI interaction with Veratric acid 3D and 2D view

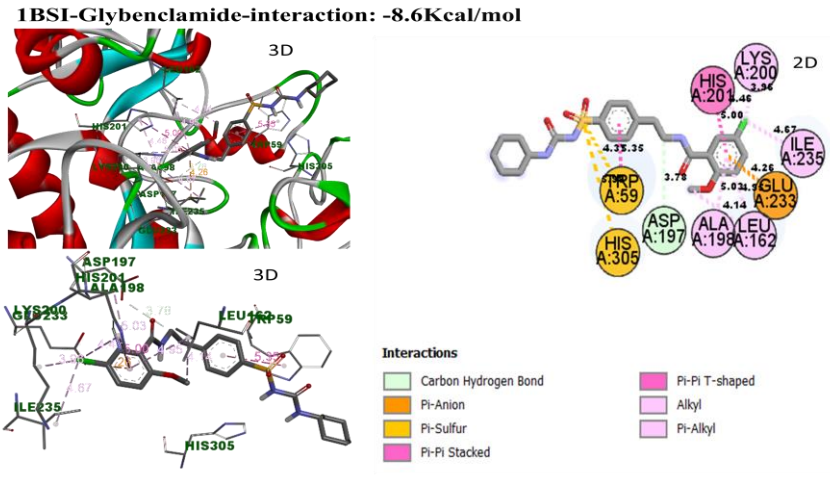


Fig. 11. PDB:1BSI interaction with glybenclamide 3D and 2D view

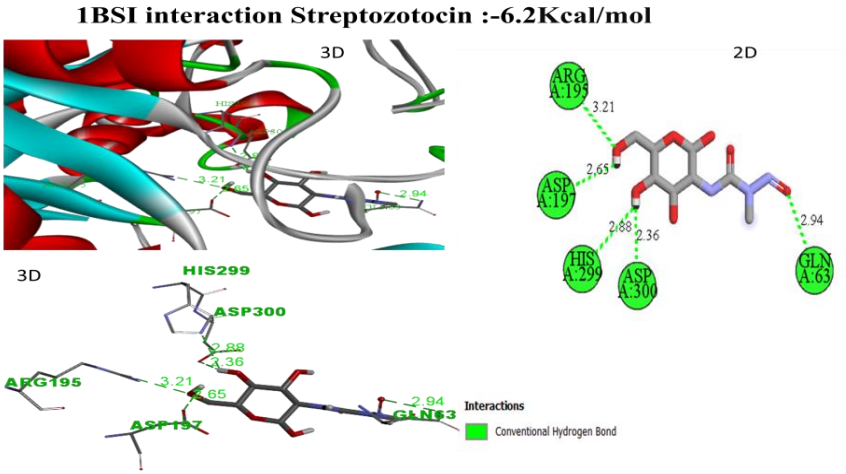


Fig. 12. PDB:1BSI interaction with Streptozotocin 3D and 2D view

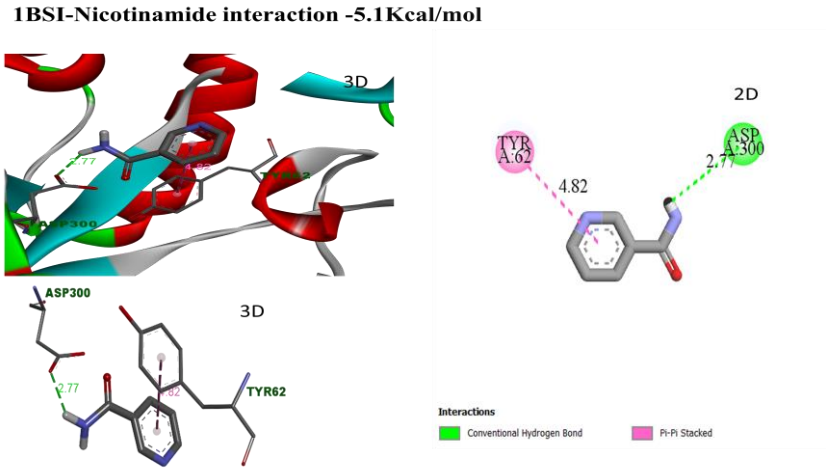


Fig. 13. PDB:1BSI interaction with Nicotinamide 3D and 2D view

The molecular docking study highlights significant interactions between the selected ligands—Veratric Acid, Glibenclamide, Streptozotocin, and Nicotinamide—and the human pancreatic enzyme (PDB ID: 1BSI). The analysis of binding energies, interaction types, and key amino acid residues provides valuable insights into the inhibitory potential of these compounds.

VeratricAcid: Veratric Acid exhibits a binding energy of -5.6 kcal/mol, forming several key interactions with the enzyme. It creates hydrogen bonds with ASP A:300 and TYR A:163 at distances of 3.08 Å and 3.00 Å, respectively. Additionally, a carbon-hydroxyl bond with ASP A:197 (3.59 Å) and Pi-alkyl interactions with LEU A:162 and ALA A:198 further stabilize its binding. These interactions suggest moderate inhibitory potential, supported by the involvement of key residues like ASP, TYR, LEU, ALA, LYS, and ILE in stabilizing the ligand-enzyme complex.

Glibenclamide: Glibenclamide demonstrates a stronger binding affinity compared to Veratric Acid, with a binding energy of -8.6 kcal/mol. It forms hydrogen bonds with ASP A:300, GLU A:233, and THR A:163, along with Pi-Pi T-shaped interactions with HIS A:201. Additional alkyl interactions with LEU A:162 and LYS A:200 contribute to its high affinity for the enzyme. The involvement of HIS, GLU, and LYS residues enhances the stability of the binding, supporting Glibenclamide's known potency as an effective antidiabetic agent.

Table 6. Protein 1BSI and ligands interactions chart

PDB ID / Name	Ligand Name	Binding Energy Kcal/mol	Nature of Interaction	AminoAcid Residue		Binding Distance (Å)
1BSI / Human Pancreatic Enzyme	VeratricAcid	-5.6	Conventional	ASP	A:300	3.08
				TYR	A:163	3.00
			Carbon Hydroxyl Bond	ASP	A;197	3.59
			Pi-Alkyl	LEU	A:162	4.10
				ALA	A:198	5.16
			Alkyl	Lys	A:200	4.10
				ILE	A:235	4.36
				ALA	A:106	4.86
	Glybenclaimde	-8.6	Conventional H bond	ASP	A:300	
				GLU	A:233	3.08
				THR	A:163	3.59
			Carbon Hydrogen Bond	ASP	A:197	3.59
			Pi-Pi T Shaped	HIS	A:201	5.05
			Alkyl	LEU	A:162	4.79
				LYS	A:200	4.10
				ILE	A:235	4.36
				ALA	A:198	5.16
				HIS	A:201	4.56
	Streptozotocin	-6.4	Conventional Hydrogen Bond	ARG	D.266	2.93
				ARG	D.328	3.02
				ASP	D.33	2.17
				ASP	D.328	2.44
				ARG	D.390	3.14
			Carbon Hydrogen Bond	ASP	C.383	3.41
			Unfavorable Acceptor	ASP	c.383	2.87
	Nicodinamide	-5.3	Carbon Hydroxyl Bond 2.2	GLY	B:350	2.67
				VAL	B:295	3.13
			Pi-Alkyl	VAL	B:289	4.92
				ALA	B:364	4.24
				PRO	B:294	4.78
			PI-PI Stacked	THR	B.252	3.90

Table 7. Protein 4PYP and Ligands interactions chart

PDB ID / Name	Ligand Name	Binding Energy Kcal/mol	Nature of Interaction	Amino Acid Residue		Binding Distance (Å)
4PYP /Crystal Structure Human glucose transporte GLUT1	Veratric acid	-6.2	Conventional Hydrogen bond	ASN	A:415	2.99
				ASN	A:415	3.13
				ASN	A:411	2.99
				TRP	A:388	3.12
			Unfavorable Donor-Donor	GLN	A:282	2.66
			Pi-Alkyl	PHE	A:72	5.17
				PHE	A:26	4.70
				TRP	A:412	4.39
			Pi-Alkyl	PHE	A:26	5.34
				TRP	A:412	5.73
				PHE	A:26	5.13
	Glybenclaimde	-10	Conventional Hydrogen bond	GLU	A:380	2.6
				TRP	A:388	2.84
			Pi-Sigma	TRP	A:412	3.98
			Pi-Alkyl	PHE	A:291	5.14
				TRP	A:412	4.98
			Pi-Pi T-shaped	PHE	A:26	4.97
				TRP	A:412	5.19
	Streptozotocin	-6.1	Conventional H bond	ASN	A:415	3.3
				GLN	A:283	3.01
				ASN	A:411	2.25
				TRP	A:412	2.93
				SER	A:80	2.59
	Nicotinamide	-4.6	Carbon Hydrogen bond	GLU	A:426	3.58
			Pi-Alkyl	LYS	A:300	4.53
			Pi-Alkyl	LEU	A:355	5.04
			Pi-Alkyl	PRO	A:431	4.96

Streptozotocin: Streptozotocin, with a binding energy of -6.4 kcal/mol, forms hydrogen bonds with ARG D.266, ARG D.328, ASP D.33, and ASP D.328, providing stable, albeit weaker, binding compared to Glibenclamide. The primary role of ARG and ASP residues in facilitating these interactions suggests moderate inhibitory potential, though it is less potent than Glibenclamide.

Nicotinamide: Nicotinamide shows the weakest binding affinity among the tested ligands, with a binding energy of -5.3 kcal/mol. It forms carbon-hydroxyl bonds with GLY B:350 and VAL B:295, as well as Pi-alkyl interactions with VAL B:289 and ALA B:364. Additionally, a Pi-Pi stacked interaction with THR B.252 (3.90 Å) contributes to its binding stability. However, the limited number of interactions indicates a reduced affinity for the enzyme, making Nicotinamide less effective compared to the other ligands.

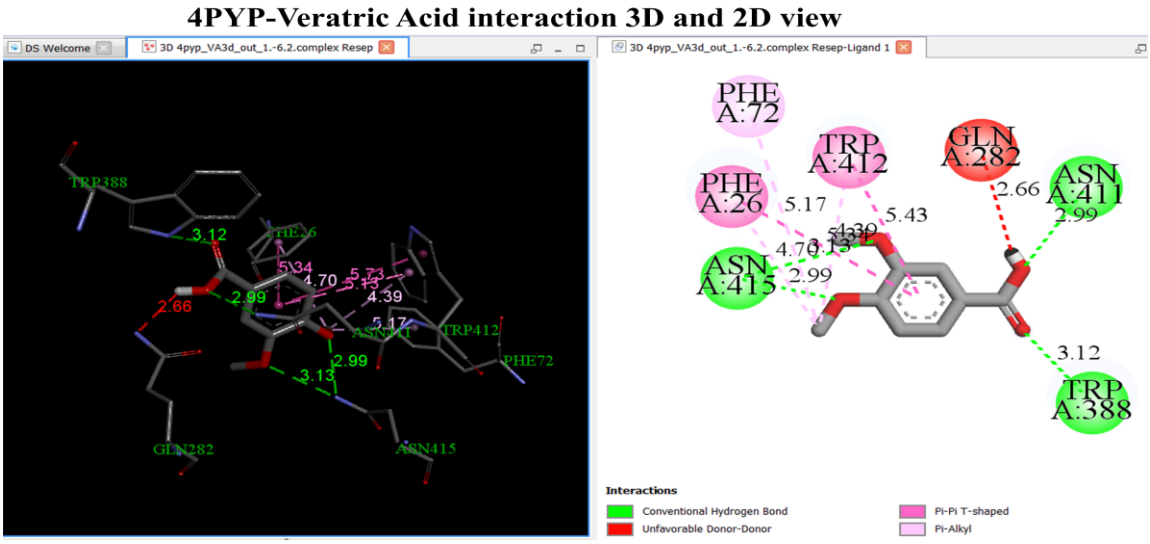


Fig. 14. PDB:4PYP Nature of interaction with Veratricacid 3D and 2D view

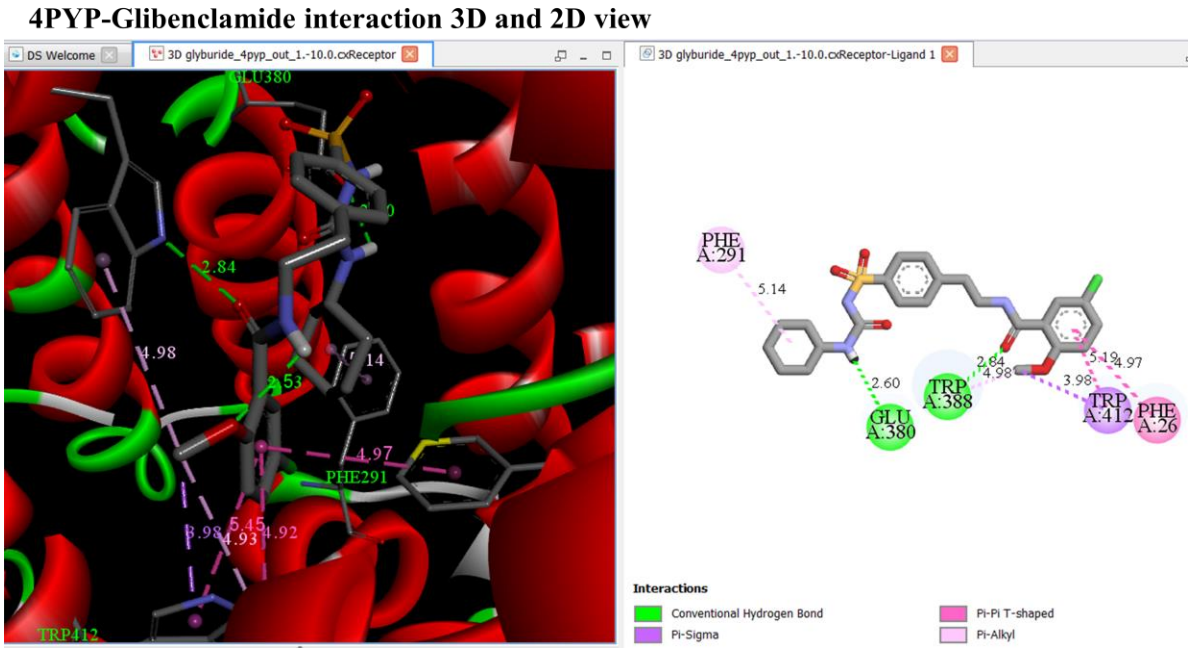


Fig. 15. PDB:4PYP Nature of interaction with Glibenclamide 3D and 2D view

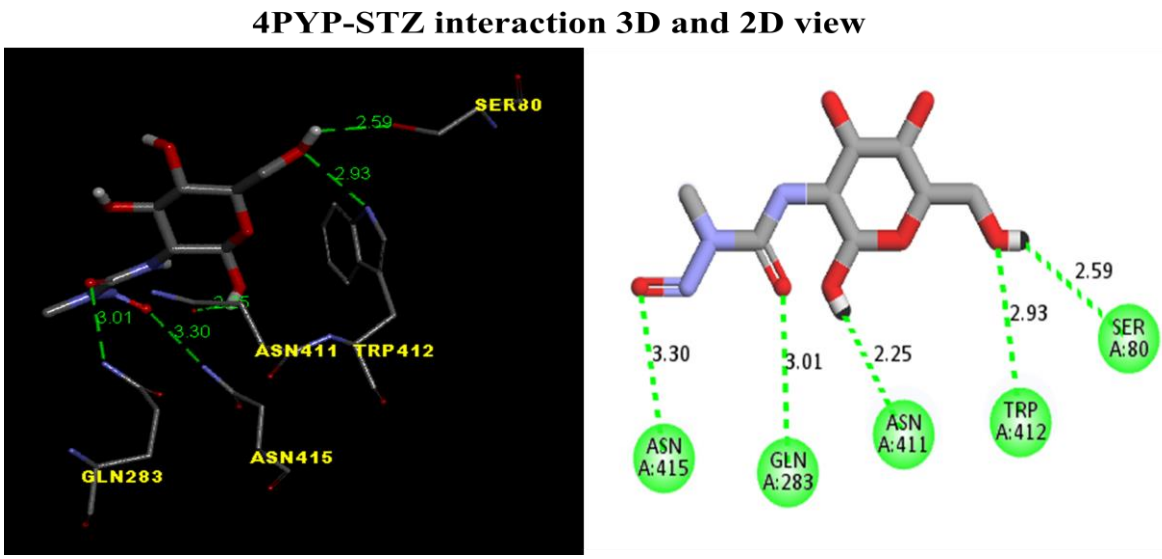


Fig. 16. PDB:4PYP Nature of interaction with Sterptozotocin 3D and 2D view

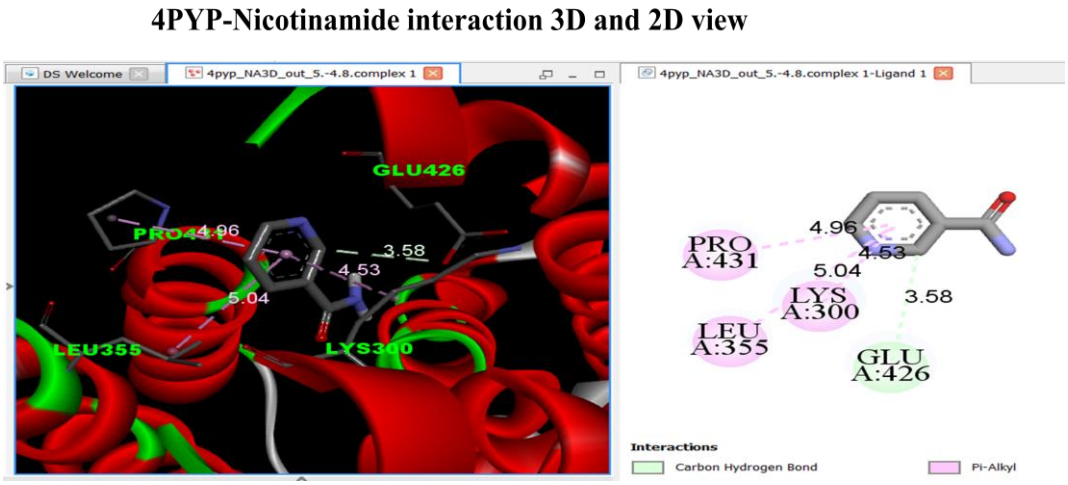


Fig. 17. PDB:4PYP Nature of interaction with Nicotinamide 3D and 2D view

The molecular docking study examines the interactions between various ligands—Veratric Acid, Streptozotocin, Nicotinamide, and Glibenclamide—with the human glucose transporter GLUT1 enzyme (PDB ID: 4pyp). The binding energies, interaction types, and key amino acid residues involved provide important insights into the inhibitory potential of these compounds.

Veratric Acid exhibits a binding energy of -5.4 kcal/mol and forms several interactions, including conventional hydrogen bonds with TYR (A:69) at a distance of 3.00 Å. It also engages in carbon-hydrogen bonds with ALA (B:30) and LYS (B:26) at 3.74 Å and 3.61 Å, respectively. Additional interactions include alkyl bonding with LYS (B:34) at 4.05 Å, Pi-alkyl interactions with ILE (B:29) and TRP (A:91) at 4.74 Å and 5.09 Å, and a Pi-sigma interaction with VAL (B:33) at 3.71 Å. These interactions suggest moderate binding affinity.

Glibenclamide demonstrates the highest binding energy at -10 kcal/mol, indicating the strongest interaction with GLUT1. It forms conventional hydrogen bonds with GLU (A:380) and TRP (A:388) at 2.60 Å and 2.84 Å, respectively. Additionally, Pi-sigma interactions with TRP (A:412) at 3.98 Å, Pi-Pi T-shaped interactions with PHE (A:26) at 4.97 Å, and Pi-alkyl interactions with TRP (A:412) at 6.35 Å further stabilize its binding, confirming Glibenclamide's potent inhibitory effect.

Streptozotocin, with a binding energy of -6.1 kcal/mol, forms conventional hydrogen bonds with ARG (A:121) at distances of 3.02 Å and 3.01 Å, and SER (A:123) at 3.00 Å. Additionally, it exhibits a donor-acceptor interaction with VAL (B:33) at 2.99 Å, indicating stronger binding compared to Veratric Acid.

Nicotinamide shows a weaker binding energy of -4.6 kcal/mol, forming conventional hydrogen bonds with TYR (A:69) at distances of 3.02 Å and 2.82 Å, as well as LEU (A:123) at 3.00 Å and 2.63 Å. The relatively few interactions reflect its lower binding affinity.

4. CONCLUSION

This study employs molecular docking and ligand interaction analysis to explore Veratric Acid as a potential antidiabetic agent, comparing its effects with established drugs like Glibenclamide on key diabetic-related proteins, specifically 1BSI and 4PYP. While Glibenclamide demonstrated the highest binding affinity, reinforcing its efficacy, Veratric Acid, though exhibiting a lower binding affinity, showed promise as a natural compound with potential as an adjunct therapy. Streptozotocin and Nicotinamide presented weaker affinities, suggesting limited standalone efficacy but possible complementary roles. This comparative analysis highlights the potential of integrating natural agents like Veratric Acid with conventional treatments to enhance therapeutic strategies in diabetes management.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

This study was approved by the Institutional Animal Ethics Committee (IAEC) of Rajah Muthiah Medical College (IAEC Proposal No. Annamalai University-IAEC-1353/2/23) and was conducted according to the Guide for the Care and Use of Laboratory Animals

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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