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In Silico Determination of *Tinospora cordifolia* Phytochemicals as Potential DPP-4 Inhibitors for Type 2 Diabetes Management

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Abstract

Background: Despite existing treatments, the prevalence of Type 2 diabetes mellitus (T2D) continues to rise globally, underscoring the need for novel therapeutic strategies. Medicinal plants like *Tinospora cordifolia* have shown potential in traditional medicine for managing various ailments, including diabetes. This study investigates the antidiabetic potential of *T. cordifolia* phytochemicals by targeting dipeptidyl peptidase-4 (DPP-4), a key enzyme in glucose metabolism. **Methods:** A library of 141 bioactive compounds from *T. cordifolia* was compiled and their structures retrieved from PubChem. Ligand preparation was conducted using the Schrödinger Suite, and the crystallographic structure of DPP-4 (PDB ID: 2HHA) was prepared for docking. Molecular docking, pharmacophore modeling, MM/GBSA binding energy calculations, and QSAR modeling were performed to assess binding affinities and predict inhibitory activities. Additionally, the QSAR-Toxicity Estimation Software Tool (TEST) was used to evaluate the toxicity profiles of the hit compounds. **Results:** Molecular docking revealed that five *T. cordifolia* compounds exhibited higher binding affinities than the standard drug rosiglitazone, with saponarin showing the highest affinity (-10.40 kcal/mol). MM/GBSA calculations confirmed favorable binding free energies, with saponarin exhibiting a ΔG_bind of -44.22 kcal/mol. QSAR modeling predicted that saponarin, astragalin, and tinosinenside had better pIC50 values (5.593 µM, 5.593 µM, and 5.659 µM, respectively) than rosiglitazone (5.059 µM). Pharmacophore modeling identified tinosinenside as having the highest fitness score (0.942). Toxicity assessment indicated that while tinosinenside showed potential for bioaccumulation, other compounds demonstrated moderate toxicity profiles. **Conclusion:** The findings suggest that saponarin, tinosinenside, and astragalin are promising candidates for DPP-4 inhibition and could be developed as novel therapeutic agents for T2D management. Further in vitro and in vivo studies are recommended to validate these computational predictions and explore the clinical potential of these phytochemicals.

Keywords: *Tinospora cordifolia*, DPP-4 inhibitors, type 2 diabetes, molecular docking, bioinformatics

Significance:

This study determined *Tinospora cordifolia* phytochemicals as potential DPP-4 inhibitors, offering promising therapeutic candidates for type 2 diabetes.

Introduction

Diabetes mellitus is a metabolic disorder characterized by dysregulation in carbohydrate, fat, and protein metabolism, leading to persistent hyperglycemia due to either insulin deficiency or insulin resistance (Omoboyowa et al., 2023). The disease is classified into type 1 and type 2 diabetes, with type 2 diabetes (T2D) accounting for over 90% of all reported cases, making it a major global health concern (Elekofehinti, 2023). T2D is a chronic condition marked by insulin resistance, resulting in elevated blood glucose levels that require long-term management through lifestyle modifications, dietary control, physical activity, and oral medications (Macalalad et al., 2023). However, there is currently no definitive cure for T2D, and untreated cases can lead to severe complications such as cardiovascular diseases, diabetic retinopathy leading to adult blindness, nephropathy, neuropathy, and lower-limb amputations (Roglic, 2016). The increasing prevalence of T2D worldwide highlights the limitations of existing treatments, underscoring the urgent need for novel therapeutic alternatives to complement current drug regimens.

Medicinal plants have played a crucial role in traditional and modern medicine due to their bioactive phytochemicals with therapeutic potential. Tinospora cordifolia, commonly known as Guduchi or Giloy, belongs to the Menispermaceae family and has been widely recognized for its pharmacological properties (Dhama et al., 2017). Studies have reported various biological activities of T. cordifolia, including anticancer (Singh et al., 2006), antihyperlipidemic (Stanely et al., 2000), and hepatoprotective effects (Bishayi et al., 2002). Additionally, T. cordifolia has demonstrated antidiabetic properties in preclinical studies. Rajalakshmi et al. (2009) reported that stem extracts of T. cordifolia exhibited antidiabetic effects in streptozotocin-induced diabetic rats, while Sangeetha et al. (2013) highlighted the role of palmatine, a phytoconstituent of T. cordifolia, in enhancing glucose uptake via GLUT-4 expression in L6 myotubes. Despite these promising findings, the precise mechanism underlying the antidiabetic action of T. cordifolia phytochemicals remains largely unexplored.

This study aims to investigate the molecular mechanism by which T. cordifolia exerts its antidiabetic effects by targeting key proteins implicated in T2D pathogenesis. One such target is dipeptidyl peptidase-4 (DPP-4), a serine aminopeptidase and transmembrane glycoprotein that plays a crucial role in glucose metabolism (Saini et al., 2023). DPP-4 deactivates incretin hormones, including glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide, which are essential for stimulating insulin secretion. Inhibiting DPP-4 can prolong incretin activity, thereby enhancing insulin release and improving glycemic control (Macalalad et al., 2023). This study employs advanced bioinformatics approaches, including molecular docking, quantitative structure-activity relationship (QSAR) modeling, pharmacophore modeling, MM/GBSA calculations, and pharmacokinetic profiling, to elucidate the potential of T. cordifolia phytocompounds as DPP-4 inhibitors. Understanding the molecular interactions between T. cordifolia phytochemicals and DPP-4 may pave the way for the development of novel plant-derived therapeutics for T2D management.

Materials and Methods

Preparation of Compounds

A comprehensive library of 141 bioactive compounds from *Tinospora cordifolia* was compiled through an extensive literature review using Google Scholar and PubChem databases (https://pubchem.ncbi.nlm.nih.gov/). The chemical structures of these compounds, along with the co-crystallized ligand and a standard reference drug, were retrieved in Structure Data File (SDF) format. Ligand preparation was conducted using the LigPrep module of Schrödinger Suite (version 2017-V2). The OPLS3 force field was applied at a pH of 7.0 ± 2, with Epik used for ionization state generation. Desalting and tautomer generation options were enabled to ensure structural integrity. Stereoisomer configurations were maintained to retain specific chiral centers while varying other centers, generating a maximum of one stereoisomer per ligand.

Target Protein Preparation and Docking Procedure

The crystallographic structure of dipeptidyl peptidase-4 (DPP-4) from *Homo sapiens* (PDB ID: 2HHA) was obtained from the RCSB Protein Data Bank (www.rcsb.org). Monomeric chain A of the protein was prepared using the Protein Preparation Wizard in Schrödinger Suite (version 2017-V2). This process involved adding missing hydrogen atoms, assigning bond orders, optimizing hydrogen-bonding networks, and minimizing energy using the OPLS3 force field. The receptor grid was generated at the binding site of the co-crystallized ligand with coordinates set at x = 39.15, y = 49.23, and z = 38.06 (Omoboyowa, 2024).

Prepared *T. cordifolia* compounds were virtually screened against the DPP-4 binding site using the Glide module in Schrödinger Suite. Extra precision (XP) docking mode was employed to ensure high accuracy in binding affinity predictions. The resulting protein-ligand complexes were visualized and analyzed using Discovery Studio Visualizer 2020. The binding interactions and energy profiles were assessed to identify the most promising inhibitors. The crystallographic structure and docking interactions are illustrated in Figure 2.

Pharmacophore Modeling and Fitness Score Estimation

Pharmacophore modeling was performed using the Receptor-Ligand Pharmacophore Hypothesis module of Schrödinger Suite (version 2017-V2). A pharmacophore hypothesis was generated based on the active site interactions of the crystallographic DPP-4 structure. This hypothesis was then used to screen the *T. cordifolia* compounds. Fitness scores were calculated using the Phase Screen module to evaluate the alignment of the compounds with the pharmacophore features (Omoboyowa, 2022).

QSAR Modeling and PIC50 Estimation

Experimental datasets of known DPP-4 inhibitors, along with their respective PIC50 values, were retrieved from the ChEMBL database (https://www.ebi.ac.uk/chembl/) through BLAST analysis of the DPP-4 FASTA sequence. The datasets were converted into SDF format using DataWarrior software and subsequently imported into the Maestro workspace of Schrödinger Suite. The MacroModel minimization tool was used to optimize the structures. A quantitative structure-activity relationship (QSAR) model was developed based on the experimental PIC50 values. This model was then applied to predict the PIC50 values of the *T. cordifolia* hit compounds to assess their inhibitory potential against DPP-4 (Omoboyowa, 2022).

Toxicity Prediction Using QSAR-TEST

The toxicity of the hit compounds was evaluated using the QSAR-Toxicity Estimation Software Tool (TEST), Version 4.2 (Martin, 2016). The primary endpoint analyzed was the oral rat LD50 (lethal dose for 50% of the population). The predicted toxicity values were classified according to the mammalian toxicity scale categories provided by the Agency for Toxic Substances and Disease Registry (ATSDR): Category X (Extreme toxicity), A (Very high toxicity), B (High toxicity), C (Moderate toxicity), and D (Low toxicity) (Sripriya et al., 2019). Additionally, the bioaccumulation factor (BF), developmental toxicity (DT), and mutagenicity of the compounds were assessed using TEST 4.2 to ensure comprehensive safety profiling.

Results and Discussion

The pursuit of novel small molecules that can effectively modulate target protein activity for therapeutic applications is the cornerstone of drug design. Identifying these chemical compounds within the vast chemical space is unattainable without prior knowledge of their molecular structures and interactions (Bodun et al., 2025). Consequently, virtual screening of natural compounds derived from medicinal plants has emerged as a critical method in discovering such bioactive molecules. In this study, bioactive compounds from *Tinospora cordifolia* were screened against dipeptidyl peptidase-4 (DPP-4), a significant therapeutic target for type 2 diabetes.

Results of Molecular Docking Study

Molecular docking, a pivotal tool in drug discovery, employs virtual screening protocols to predict the binding model and affinity of chemical compounds within the binding sites of protein targets (Omoboyowa, 2024). Validation of docking procedures is crucial to ensure the reliability and reproducibility of the protocols. In this study, the co-crystallized ligand from the protein's crystallographic structure was re-docked into its binding domain for superimposition, as illustrated in Figure 2. The root mean square deviation (RMSD) of the superimposed structure was 1.992 Å, falling below the acceptable threshold of 2.0 Å, thereby confirming the protocol's reliability (Balogun et al., 2021).

Molecular docking elucidates interactions between small molecules and proteins at the atomic level, highlighting the binding affinity of protein-ligand complexes. Among the 141 natural compounds from *T. cordifolia* screened against DPP-4, five exhibited higher binding affinities than the standard drug, rosiglitazone. Rosiglitazone, a potent member of the thiazolidinedione class, is commonly used alongside other anti-diabetic medications like metformin to enhance the body's insulin sensitivity and regulate blood sugar levels. The results presented in Figure 2 indicate that saponarin demonstrated the highest binding affinity (-10.40 kcal/mol), surpassing the co-crystallized ligand's binding affinity of -9.74 kcal/mol.

This superior binding affinity is attributed to the interaction of various functional groups within these compounds and the amino acid residues at the DPP-4 binding site. As depicted in Figure 4, saponarin exhibited the highest number of hydrogen bond interactions among the hit compounds and standards, forming seven hydrogen bonds with ARG 125, GLU 205, HIS 126, CYS 551, and ASP 545. Tinosinenside followed closely with six hydrogen bonds involving ARG 669, GLU 206, TYR 662, ARG 125, and GLU 205. The co-crystallized ligand formed five hydrogen bonds with ARG 356, ARG 669, ARG 125, and ASN 710. Other hit compounds exhibited fewer hydrogen bonds: astragalin formed four, tyramine one, and higenamine none. Although other interaction types such as salt bridges, pi-sulfur, and carbon-hydrogen bonds were observed (Figure 5), hydrogen bond formation plays a critical role in stabilizing the three-dimensional structure of protein-ligand complexes (Pace et al., 2014).

To validate the binding energies of the hit compounds, Molecular Mechanics Generalized Born Surface Area (MM/GBSA) calculations were conducted. MM/GBSA is a widely used method for biomolecular studies, including protein folding, protein-ligand binding, and protein-protein interactions. The binding free energy values (ΔG_bind) presented in Figure 3 showed that the standard drug (rosiglitazone) and the co-crystallized ligand had the most favorable MM/GBSA scores of -58.25 and -51.79 kcal/mol, respectively. Among the hit compounds, saponarin exhibited the best binding free energy of -44.22 kcal/mol, while tyramine had the least favorable score of -18.06 kcal/mol.

PIC50 Prediction of Hit Compounds via QSAR Modeling

Quantitative Structure-Activity Relationship (QSAR) modeling is a computational technique that establishes correlations between the biological activities of chemical compounds and their structural properties. The principle underpinning QSAR is that variations in structural attributes result in differing biological activities (Kwon et al., 2019). In this study, AutoQSAR modeling of DPP-4 inhibitors retrieved from the ChEMBL database was performed. The dataset was automatically partitioned into 75% training and 25% test sets. The best-performing model, identified as kpls_molprint2D_36, was selected to predict the biological activities of the hit compounds. Model parameters are summarized in Table 2, and the observed versus predicted activities for the training and test sets are illustrated in the scatter plot in Figure 5.

Based on predicted activity (pIC50) values, the co-crystallized ligand, with a pIC50 of 6.054 µM, was predicted to be more active than the hit compounds. However, saponarin, astragalin, and tinosinenside, with pIC50 values of 5.593 µM, 5.593 µM, and 5.659 µM respectively, demonstrated better predicted activity than the standard drug rosiglitazone, which had a pIC50 of 5.059 µM (Table 3).

Pharmacophore Modeling and Phase Screening of Bioactive Compounds

Pharmacophore modeling is a cutting-edge technology utilized to identify and characterize the potential interactions within ligand-receptor complexes. These interactions encompass steric and electronic features essential for eliciting a biological response (Tyagi et al., 2022). In this study, an E-pharmacophore model was generated to elucidate the steric

properties of the co-crystallized ligand critical for optimal interaction with DPP-4. This pharmacophore hypothesis was subsequently applied to screen the hit compounds for shared steric features with the co-crystallized ligand.

By comparing these features and evaluating their similarity, fitness scores for the hit compounds and the standard drug were calculated. The pharmacophore hypothesis for the co-crystallized ligand's optimal interaction with DPP-4 comprised four features, as depicted in Figure 6. Fitness scores for the hit compounds are presented in Table 4. Tinosinenside exhibited the highest fitness score of 0.942, with three out of four features matched. The standard drug rosiglitazone followed with a fitness score of 0.564, matching all four features. Astragalin and higenamine matched all four features but had lower fitness scores of 0.476 and 0.433, respectively. Higher fitness scores indicate a stronger predicted biological activity of the compound against the target protein.

Overall, the findings from molecular docking, MM/GBSA binding energy calculations, QSAR modeling, and pharmacophore screening provide a comprehensive understanding of the potential anti-diabetic properties of *T. cordifolia* bioactive compounds. The results highlight saponarin, tinosinenside, and astragalin as promising candidates for further investigation and development as therapeutic agents for type 2 diabetes.

Results of the Toxicity Prediction by QSAR-TEST

The toxicity assessment was conducted using the Quantitative Structure-Activity Relationship (QSAR) Toxicity Estimation Software Tool (TEST), a computational model designed to predict the potential toxicity of chemical compounds based on their molecular structure and properties. This tool facilitates the estimation of various toxicity endpoints, including bioaccumulation, developmental toxicity, mutagenicity, and lethal dose (LD50) values.

As presented in Table 5, among the screened compounds, *tinosinenside* exhibited a high bio-concentration factor (BCF) of 146.22, indicating a greater potential for bioaccumulation in living organisms. In contrast, other small molecules demonstrated moderate bioaccumulation potential, with the exception of *saponarin*, for which the QSAR-TEST tool did not provide a prediction. The bio-concentration factor represents the ratio of a chemical's concentration within an organism to its concentration in the surrounding environment at steady state, serving as a critical indicator of a substance's bioaccumulation potential (Petoumenou et al., 2015). Thus, *tinosinenside* may exhibit slight bioaccumulation in organisms, whereas the other compounds are more likely to be efficiently cleared from biological systems.

Developmental toxicity, defined as the potential of a chemical to interfere with the normal development of an organism due to exposure either before conception or during development, was also evaluated (ECHA, 2017). *Higenamine* and *astragalin* were predicted to have developmental toxicity values of 0.76 and 0.58, respectively, classifying them as toxic according to the FDA/TERIS database (Sussman et al., 2003). This suggests that these compounds may pose developmental risks upon exposure.

Regarding mutagenicity, only *tyramine* demonstrated a high mutagenicity value of 0.56, indicating a significant potential to cause genetic mutations. Mutagenicity is a crucial factor in assessing the long-term genetic risks posed by chemical compounds.

The predicted lethal dose (LD50) values in oral rats, which measure the dose required to cause death in 50% of the test population, revealed that *tinosinenside* exhibited moderate toxicity. In contrast, the remaining compounds, including *saponarin*, *higenamine*, *astragalin*, and *tyramine*, were predicted to have low toxicity levels, indicating a safer profile for potential therapeutic use (Table 5).

Overall, the QSAR-TEST predictions provide valuable insights into the safety profiles of the bioactive compounds from *T. cordifolia*. While *tinosinenside* shows some bioaccumulation and moderate toxicity, the other compounds demonstrate favorable toxicity profiles, supporting their potential as safer candidates for further investigation in diabetes treatment.

Conclusion

This study demonstrates the potential of *Tinospora cordifolia* phytocompounds as therapeutic agents for type 2 diabetes (T2D) through targeted inhibition of dipeptidyl peptidase-4 (DPP-4). Using advanced bioinformatics approaches, including molecular docking, QSAR modeling, MM/GBSA calculations, and pharmacophore modeling, saponarin, tinosinenside, and astragalin emerged as promising candidates with superior binding affinities and favorable pharmacokinetic profiles compared to the standard drug rosiglitazone. Saponarin exhibited the highest binding affinity (-10.40 kcal/mol) and demonstrated significant interactions with key amino acid residues in DPP-4. Additionally, toxicity assessments via QSAR-TEST indicated minimal bioaccumulation and acceptable safety profiles for most hit compounds, with tinosinenside showing slight bioaccumulation potential. These findings underscore the promise of *T. cordifolia* in developing novel plant-based therapeutics for T2D management. However, further in vitro and in vivo validation is essential to confirm these bioactivities and ensure clinical applicability.

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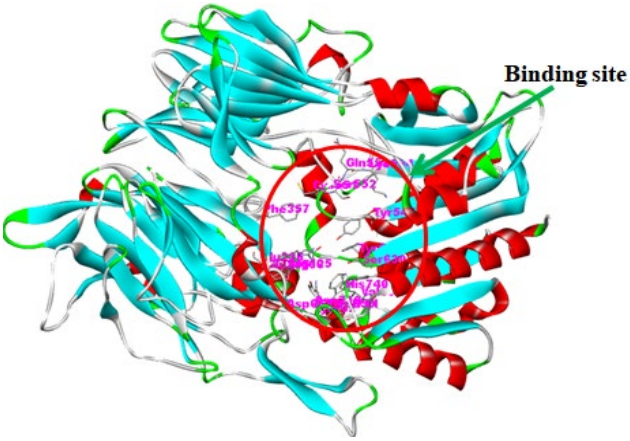

Figure 1: Crystal structure of DPP-4 (2HHA)

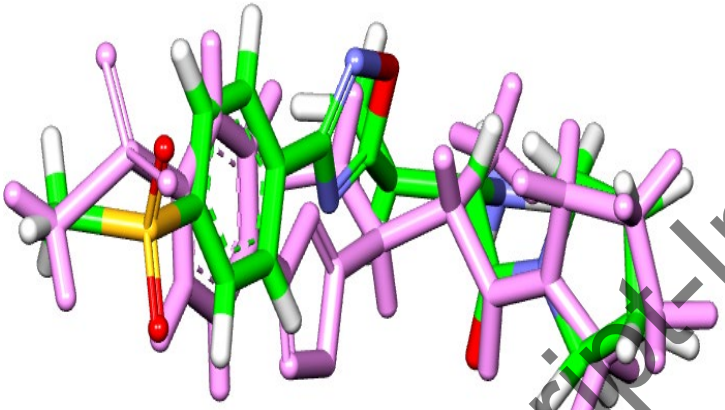

Figure 2: Validation of docking procedure by superimposition of the co-crystallized ligand

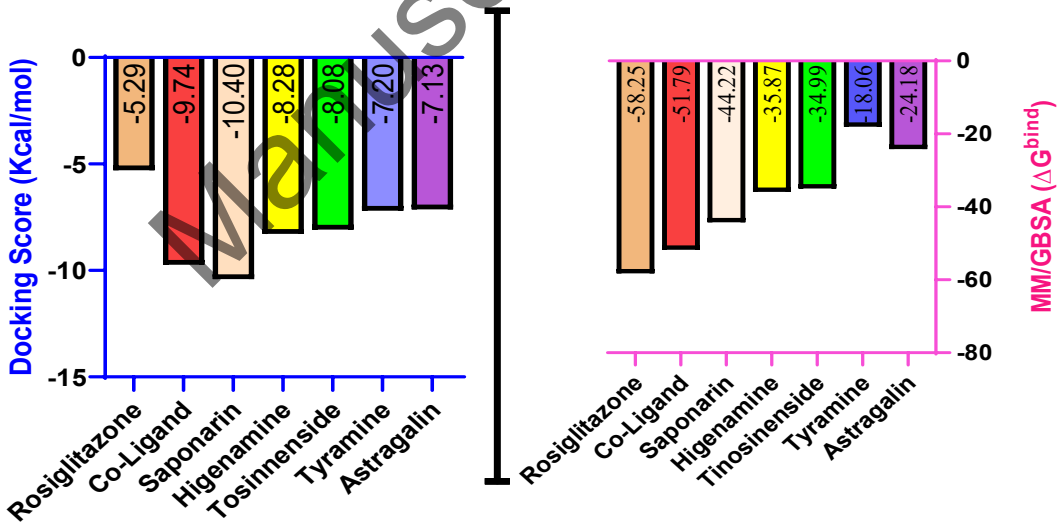

Figure 3: Representation of the binding affinity and MM/GBSA (ΔGbind) of hit molecules

Table 1: Binding interactions of hit compounds against target of diabetes

Compounds ID	No of H-bond	Interacting Residues
Rosiglitazone	2	GLN 553; TYR 666
Co-Ligand	5	ARG 356; ARG 669; ARG 125; ASN 710
Saponarin	7	ARG 125; GLU 205; HIS 126; CYS 551; ASP 545
Astragalin	4	ASN 710; TYR 662; GLU 206; GLN 553
Higenamine	NIL	NIL
Tinosinenside	6	ARG 669; GL;U 206; TYR 662; ARG 125; GLU 205
Tyramine	1	GLU 205

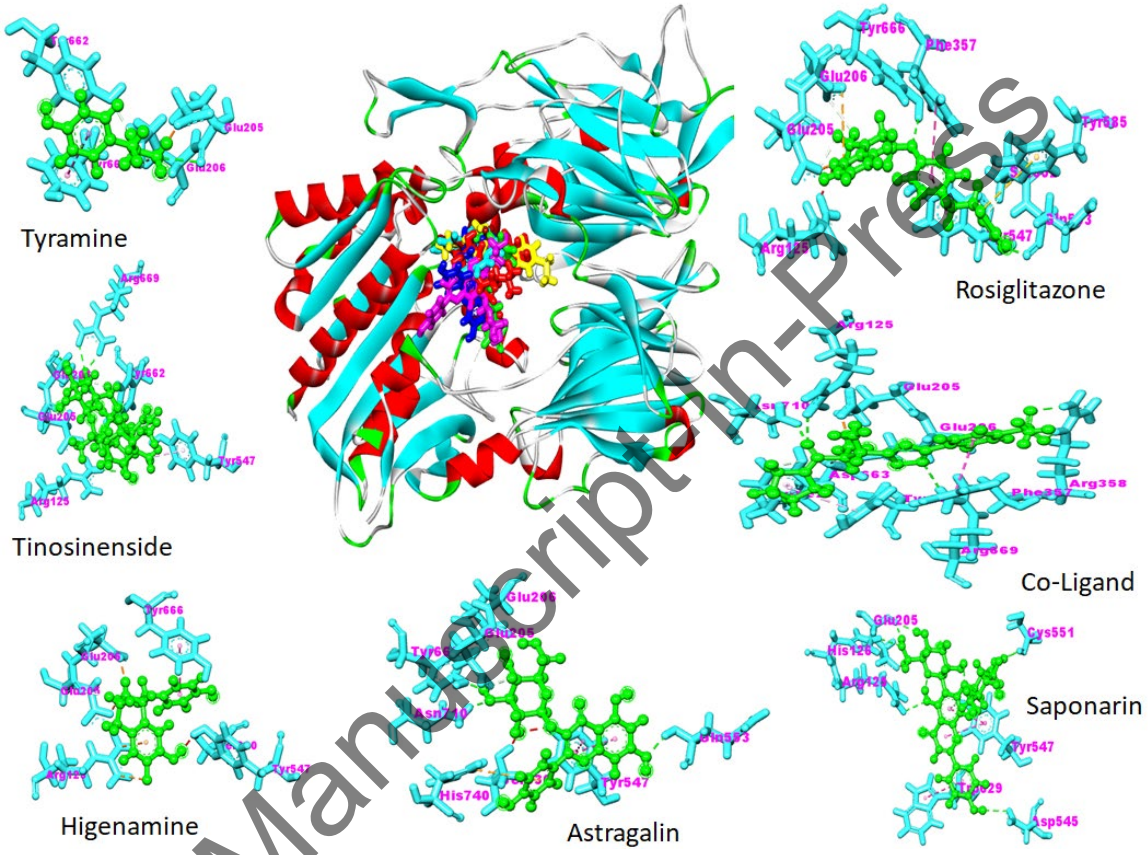

Figure 4: Interactions of the hit compounds and amino acid residues at DPP-4 binding site

Table 2: Parameters corresponding to the selected model

Best Model	S.D	R^2	RMSE	Q^2
kpls_molprint2D_36	0.5180	0.7823	0.6136	0.5487

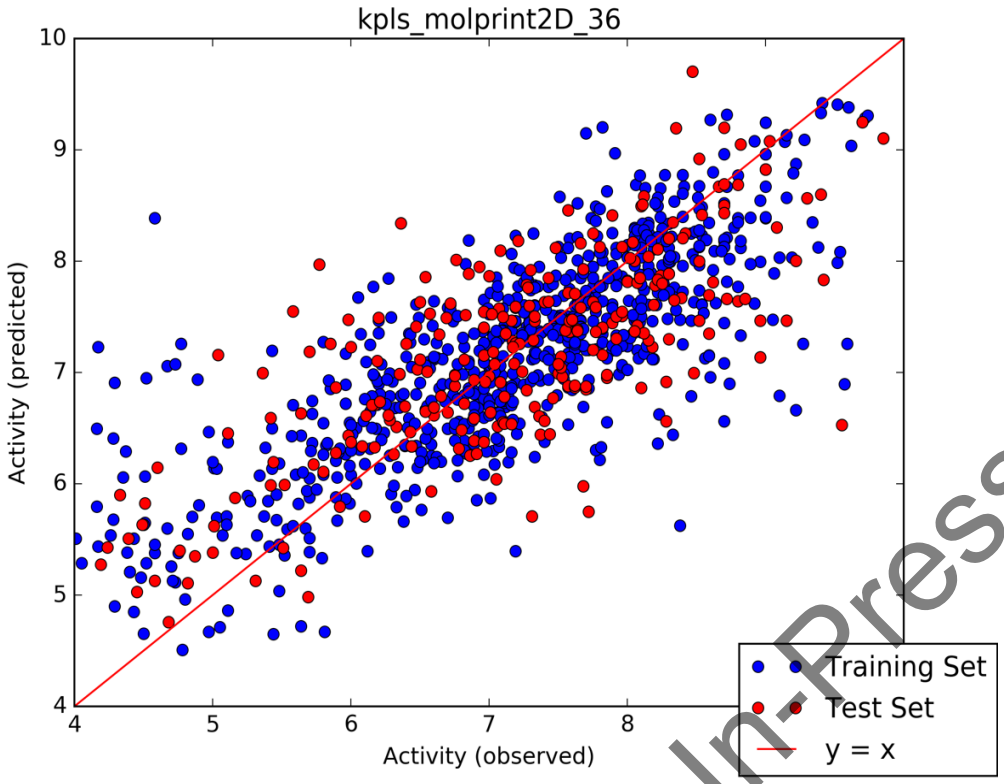

Figure 5: Scatter plot of the observed and predicted activity

Table 3: PIC50 of hit compounds

Compound ID	pIC50 (μm)
Rosiglitazone	5.059
Co-Ligand	6.054
Saponarin	5.593
Astagalin	5.593
Higenamine	5.036
Tinosineneside	5.659
Tyramine	4.863

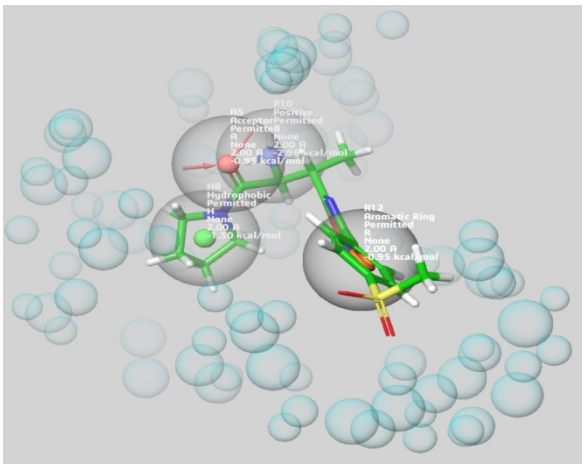

Figure 6: E-pharmacophore hypothesis between co-crystalized ligand and DPP-4

Table 4: Fitness score of hit compounds

Compound ID	Matched Ligand sites	Fitness score
Rosiglitazone		0.564
Co-Ligand		0.452
Saponarin		0.412
Astragalin		0.476
Higenamine		0.433
Tinosinenside		0.942
Tyramine		0.455

Table 5: Prediction of toxicity parameters of the hit compounds

Compound ID	Bio-concentration Factor	Developmental Toxicity		Mutagenicity		Oral Rat	
		Value	Result	Value	Result	LD_{50} (mg/kg)	Category
Saponarin	NA	0.40	DNT	0.12	-ve	3433.94	D
Higenamine	9.48	0.76	DT	0.47	-ve	1895.65	D
Tinosinenside	149.22	0.42	DNT	0.08	-ve	33.90	C
Tyramine	2.59	0.44	DNT	0.56	+ve	1170.73	D
Astragalin	13.77	0.58	DT	0.38	-ve	2576.37	D

DT: Developmental toxicant; DNT: Developmental Non-toxicant; -ve: Mutagenicity negative; +ve: Mutagenicity positive; NA: Not applicable; C: Moderate toxicity; D: Low toxicity