In-silico ADMET analysis for the Best Natural DPP-4 inhibitors as a potential Antidiabetic Agent

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ABSTRACT

Background:
Diabetes mellitus is the major health problem that affects majority of the population. Among Type 2 Diabetes mellitus treatment strategies, DPP-IV enzyme inhibition has been proven to be best effective method. The natural secondary metabolites offer the advantage over the synthetic one.

Methods:
In our study, we have done in-silico ADME-T studies using Swiss ADME, Pro-TOX-II and PreADMET of the 15 natural secondary metabolites i.e., N-Nororientaline, Cyanidin 3,5-diglucoside, Diprotin A, Amentoflavone, Stigmasterol, 7-deoxy-6-epi-castanospermine, Robinin, Rutin, Antroquinonol, Curcumin, Calebin A, Quercetin, Puromycin, 16-hydroxycleroda-3,13-dien-15,16-olide (HCD), and Epigallocatechin gallate.

Results:
The study projected that Diprotin A and 7-deoxy-6-epi-castanospermine are the substances with the best pharmacokinetic profiles and the least amount of systemic toxicity.

Conclusion:
This evaluation offers valuable insight into the future creation of innovative medications for the management of diabetes mellitus.

Keywords: Diabetes, Dipeptidyl peptidase, secondary metabolites, *in-silico* study, ADME-T studies

Graphical Abstract-

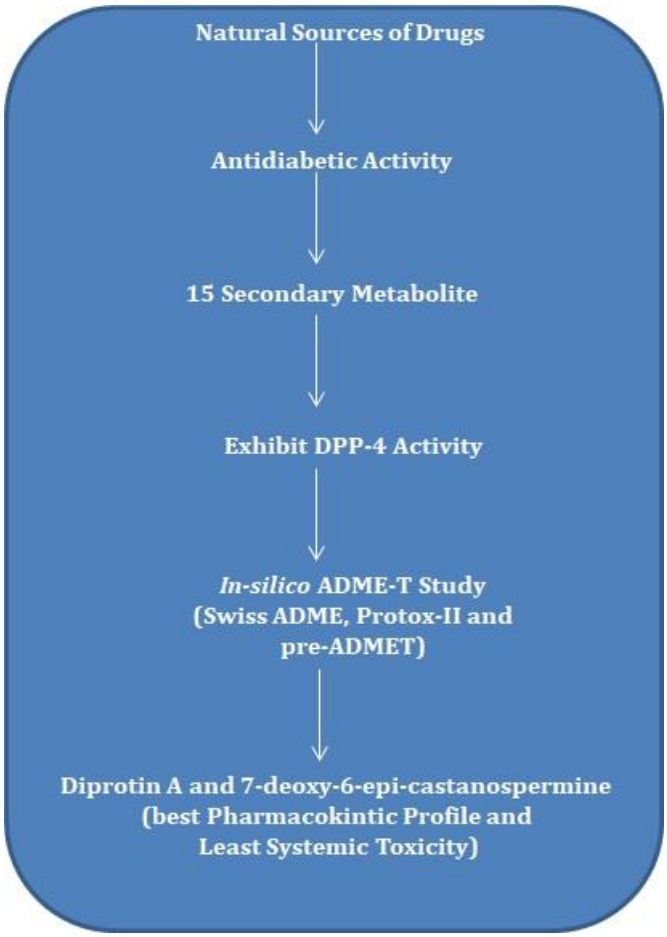

Figure-1 - Natural Sources of Drugs: Antidiabetic Activity

1. INTRODUCTION

Diabetes can be defined as the chronic metabolic disorder that cases the elevated blood glucose levels. According to World Health Organization, presently 422 million population have diabetes. Diabetes causes approximately 1.5 million deaths per year. It causes serious damage to organs such as heart, eyes, kidneys, blood vessels and nerves. The major symptoms of the diabetes are hyperglycemia (elevated blood glucose levels), polydipsia (excess thirst), polyuria (excess urination), polyphagia (excessive eating), weight loss, weak eye side, diabetic foot (nerve damage in feet), ketoacidosis (increased production of ketones) and non-ketonic hyperosmolarity (increased serum osmolarity). Capillary basement membrane thickening, an increase in vessel wall matrix, and cellular proliferation are common pathological changes that cause vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy, and peripheral vascular insufficiency. There are three types of diabetes i.e., Type 1 diabetes, Type 2 diabetes and gestational diabetes. Type 1 diabetes is also knowing as insulin dependent diabetes or juvenile diabetes. Type 2 diabetes is known as insulin independent diabetes or maturity onset diabetes. Gestation diabetes occur in the case of pregnancy.[1]

1.1. PATHOPHYSIOLOGY

Type I diabetes

It affects approximately 10% of the population that are diagnosed with diabetes. Type I diabetes I as insulin dependent diabetes. In this type, disease develops due to the insufficiency of the hormone insulin. Insulin is released form the β- cells of the islets of Langerhans of the pancreas. Insulin is responsible for maintaining the blood glucose levels. It is a type of autoimmune disorder. The immune cells destroy the insulin secreting cells. The anti-insulin antibodies are responsible for action. The factors that are responsible for the T1DM are genetic factors (HLA, Insulin-VNTR, CTLA-4, PTPN22, AIRE, FoxP3, STAT3, IFIH1, HIP14 and ERBB3), epigenetic factors, and immunological factors (immune tolerance, cellular immunity or humoral immunity). [2]

Type II diabetes

It is also known as non-insulin dependent diabetes or adult-onset diabetes. The major cause of the type II diabetes is the insufficient production of insulin by the β-cell of the islets of Langerhans or loss of the insulin sensitivity by the cells (reduction in the insulin receptors on cells). The development of this type of diabetes is highly associated with the obesity BMI ≥ 30 kg/m^2. The factors that include development of this includes adipokine dysregulation, inflammation, abnormalities in gut microflora, or immune dysregulation. The development of the type II diabetes includes inflammatory damage such as elevated level of free fatty acid and adipokine dysregulation which results in the loss of the insulin sensitivity or insulin resistance in the adipose tissues. Activation of apoptotic unfolded protein pathway is the best explained mechanism for the enhanced of the ER stress by the elevated free fatty acid levels. The metabolic stress is another reason for the β-cell damage and reduced secretion of insulin. Insulin resistance reduces the uptake of the glucose by muscles, liver and adipose tissue. This generates the condition on imbalance in the demand and supply. The reduced uptake of the glucose by cells causes elevated blood glucose levels i.e., hyperglycaemia. T2DM is a genetic based disorder. The improved can be best preventive method for the T2DM.[3]

Gestational diabetes

The elevated levels of the glucose, hyperglycaemia that develops during the pregnancy are medically termed as the gestational diabetes. The major factors that fasten the development of the gestational diabetes includes micronutrients deficiency, improper diet during pregnancy, obesity, age or the family history of diabetes. The consumption of high saturated fat diet interferes with the insulin signalling. In most cases, GDM conditions reverses after the delivery bur in some it increases the risk of T2DM in mother and risk of diabetes and overgrowth of the foetus. The major reason that has been reported for the GDM is the loss of the insulin sensitivity or increases insulin resistance (cells not responding to insulin) by the cells and β-cell dysfunction Ing. During the pregnancy, the glucose levels are elevated in order to supply energy to the placenta and foetus this causes prolonged and excessive insulin production by the β-cells. This prolonged and excessive production results in β-cell dysfunction i.e., loss of the ability to responses against the elevated blood glucose levels.

During the pregnancy several hormones are secreted by the glands to maintain the healthy gestation period. There hormones such as estrogen, progesterone, cortisol, leptin, placental lactogen or placental growth hormones promotes the insulin resistance in the adipose tissue, liver and muscles cells to increase the blood glucose level in maternal blood. This glucose is further utilizing for the growth and development of the foetus. All these are the mechanism that resulted in GDM. [4]

1.2 Diabetes treatment strategies

The treatment strategies for the diabetes types differs according to the pathophysiology. In case of the type I diabetes the β-cell number falls due to the cell death caused by the self-immune cells. The patient pancreatic cells lose the ability to synthesise and secret the endogenous insulin. The exogenous insulin administration is he best suited treatment option for the management of T1DM. There are several ways to optimise metabolic function while using insulin therapy. Basal insulin levels can be maintained by a long-acting insulin analogue. Pre-meal administration of rapid-acting insulin provides better control to the blood glucose levels. [5] The oral hypoglycaemic agents (OCA) have proven to be the best suited method for the treatment of the T2DM and gestational diabetes. This includes drugs that enhances the insulin secretion such as sulfonylureas and DPP-IV inhibitors, drugs that overcome insulin resistance such as biguanides and thiazolidinediones, drugs that reduces carbohydrate absorption such as α-glucoside inhibitors and certain miscellaneous drugs categories such as SGLT-2 inhibitors and Dopamine D2 agonists.[6]

1.3 DPP-IV and its inhibitors

Dipeptidyl peptidase (DPP-IV) is a glycoprotein of size 100kDa related to the prolyl oligopeptidase family. It is also designated as CD26.Chromosme 2 carries the gene for human DPP.[7] It is responsible for the cleavage of the peptide chain from the N-terminal hence categorised as exopeptidase. It is found in two forms i.e., DPP-IV and sDPP. DPP-IV enzymes are found on surface of the cells of intestine, vascular endothelium, liver, pancreas, glands and certain immune cells such as fibroblast or leukocytes.[8] sDPP is the soluble form the enzymes that is found freely int the blood. DPP-IV enzymes belong to the type II transmembrane protein family. Its structure consists 4 portions i.e., short cytoplasmic domain, transmembrane domain, flexible stalk segment and extracellular domain. sDPP form doesn't have the cytoplasmic and transmembrane domain. sDPP in produced by the action of MMP's on the cellular DPP-IV enzyme by the process of shedding.[7]

DPP-IV enzyme plays as important role in the glucose homeostasis in the type II diabetes. The inhibit the activity of the incretin hormones such as GLP-1 (glucagon like peptide-1) and GIP (gastric inhibitory peptide). GLP-1 and GIP are involved in the lowering of the blood glucose levels by stimulating the β-cells to increase insulin secretion, reducing glucagon levels and delaying gastric emptying. DPP-IV enzyme inactivates these peptides by its exopeptidase action. DPP-IV inhibitors inhibit the activity of this enzyme and hence increase the levels of GLP-1 and GIP. As a result, the insulin secretion by the β-cells of pancreas reschedules which re-establishes the blood glucose levels.

This property of DPP-IV inhibits makes it the best suited therapy for the treatment of T2DM.[9] Since ancient times, medicinal plant use has been widespread in India. The Rigveda, the Yajurveda, the Samaveda, and the Atharvaveda are the four Vedas that make up Indian civilization. The Atharvaveda, which includes the upaveda known as Ayurveda, is one of these Vedas. Many common plants have been classified as having therapeutic qualities in Ayurveda. These drugs are believed to have minimal or no negative effects. Due to the presence of the phytoconstituents, these plants have anti-diabetic, neuroprotective, anti-inflammatory, and antioxidant activities. Plant compounds that are bioactive or chemically active are known as phytoconstituents or secondary metabolites. There have been many active chemicals discovered, and they have been divided into 16 major categories. Alkaloids, terpenoids, phenols, phenolic glycosides, coumarins, their glycosides, anthraquinones, flavones, and flavonoid glycosides, as well as mucilage and gums, tannins, volatile oils, and anthraquinones. I this research we have presented the ADME-T studies of the 15 secondary metabolites found in different plant species that exhibits the anti-diabetic property via DPP-IV inhibition.

1.4 ADME-T studies

The *in-silico* ADMET studies provide the insight for the absorption, distribution, metabolism, elimination and toxicity profiles of the compound. The in-silico study offers the advantages over the in-vivo or in-vitro studies. These studies help in reducing the time for the lead discovery. The in-silico studies also reduce the cost of the research. The ADME-T studies includes various parameters such as H-bond donors, H-bond acceptors, lipophilicity, Log S value, Log P value, GI absorption, BBB penetration, P-glycoprotein substrate, cytochrome P450 inhibition, pains and brenks, leadlikeness, Lipinski rule, LD_{50} dose, toxicity class, mutagenicity, carcinogenicity, cytotoxicity, immunotoxicity, effects on TOX-21 pathways and hERG inhibition.

The Lipinski rule of 5 includes (molecular weight ≤500, number of hydrogen bond donors ≤5, number of hydrogen bond acceptors ≤10, and lipophilicity (Log P) ≤5. According to this rule an orally active drug should posses not more than 1 violation to qualify for the rule. [10] Lipophilicity of the molecule has been always the crucial part in the drug discovery. Lipophilicity (Log P) is defined as the partition coefficient of the molecule in octanol and water. The numerous processes in which lipophilicity (or log P) of molecules plays a significant role are drug dissolution in the gastrointestinal tract, intestinal absorption, permeation into the portal vein, first pass through the liver, pharmacokinetic properties, hydrophobic drug-receptor interactions, as well as toxicological properties of drugs.[11]

Log S values determine the aqueous solubility of the compound. Aqueous solubility of the compound helps in determine the fate of movement and excretion of the molecule from the body.[12] BBB penetration value determines the ability of the drug to cress Blood-Brain barrier to enter the brain. P-glycoprotein (p-gp) are the ABC (ATP-binding cassette) transporters that acts to remove the compounds or toxins out of the cells. P-glycoprotein limits the absorption of pharmaceuticals into brain cells and intestinal epithelial cells from blood circulation.

The p-gp substrate value identifies the binding of the molecule with the p-gp. [13] Leadlikeness presents the chance of the molecule to become active orally in respect to the bioavailability. Pains (pan assay interference) and brenks give out the structural alert. Pains alert arises for compounds that may give false positive result. The alert represents the compound may show non-specific binging along with the binding with the target. Brenks alert shown the metabolically instability of the compound due the presence of some moieties in the structure.[14]

The *in-silico* toxicity studies determine the carcinogenic (caner causing), mutagenic (DNA damaging ability), cytotoxic (toxic to cells), immunotoxic (adverse effects on the immune system) potential of the compound. LD_{50} dose represents the lethal dose and classifies the drug in the toxicity scale. The hERG gene (the human Ether-à-go-go-Related Gene) encodes for a protein called Kv11.1, the alpha subunit of a potassium ion channel. The hERG inhibition represents the cardiotoxicity produced by any compound. [15]

2. MATERIALS AND METHODS

2.1 Materials

The structure of the 15vsecondary metabolites of different plant species exhibiting DPP-IV inhibit activity were taken from PubChem and redrawn using ChemDraw. The selected 15 secondary metabolites are listed below.

Table 1: Compound with their Biological source

S.No	COUMPOUND	BIOLOGICAL SOURCE	FAMILY
1	N-Nororientaline	*Erythrina variegata*	Fabaceae
		Erythrina arborescens	Fabaceae
		Erythrina poeppigiana	Fabaceae
		Erythrina indica	Fabaceae
		Erythrina crystagalli	Fabaceae
2	Cyanidin 3,5-diglucoside	*Aronia arbutifolia*	Rosaceae
3	Diprotin A	*Bacillus cereus*	Bacillaceae
4	Amentoflavone	*Antidesma madagascariense*	Euphorbiaceae
5	Stigmasterol	*Urena lobata*	Malvaceae
6	7-deoxy-6-epi-castanospermine	*Castanospermum austral*	Fabaceae
7	Robinin	*Pueraria tuberosa*	Fabaceae.
8	Rutin	*Fagopyrum esculentum*	Polygonaceae
		Ruta graveolens	Rutaceae
		Sophora japonica	Fabaceae
9	Antroquinonol	*Antrodia cinnamomea*	Fomitopsidaceae
10	Curcumin	*Curcuma Longa*	Zingiberaceae
11	Calebin A	*Curcuma Longa*	Zingiberaceae
12	Quercetin	*Apium graveolens*	Apiaceae
		Morus alba	Moraceae

		Camellia sinensis	Theaceae
		Coriandrum sativum	Apiceae
		Allium cepa	Liliaceae
		Asparagus officinalis	Asparagaceae,
		Prunus domestica	Rosacea
13	Puromycin	*Streptomyces alboniger*	Streptomycetaceae
14	16-hydroxycleroda-3,13-dien-15,16-olide (HCD)	*Polyalthia longifolia*	Annonaceae
15	Epigallocatechin gallate	*Camellia sinensis*	Theaceae

2.2 *In-Silico* ADME-T studies

For the prediction of the ADME of the molecule, SwissADME was used. The software provides the effective and valuable prediction of the ADME profile of the molecule. These includes molecular formula, molecular weight, H-bond donors H-bond acceptors, lipophilicity, Log S value, Log P value, GI absorption, BBB penetration, P-glycoprotein substrate, cytochrome P450 inhibition, pains and brenks leadlikeness and Lipinski rule.

For toxicity studies PRO-TOX II and Pre-ADMET were used. This includes LD_{50} dose, toxicity class, mutagenicity, carcinogenicity, cytotoxicity, immunotoxicity, effects on TOX-21 pathways and hERG inhibition. The ADME-T prediction of all 15 molecules have been predicted as per the refence guide of these software's.

3. RESULTS AND DISCUSSION

Using in silico methods, the pharmacokinetic and toxicity predictions of the fifteen compounds that were chosen and have been scientifically demonstrated to be possible natural DPP-IV inhibitors were evaluated. Table 17 lists compounds' projected ADME characteristics. In Table 18, toxicological forecasts are listed.

3.1 N-Nororientaline

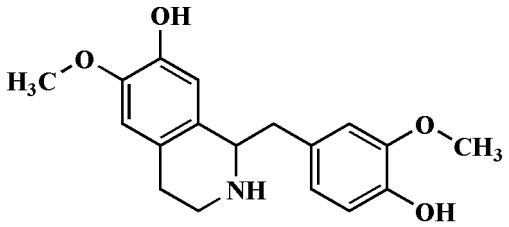

Figure-2- Structure of N-Nororientaline

Table: 2 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	315.36 g/mol
2	Number of hydrogen bond donors	≤5	3
3	Number of hydrogen bond acceptors	≤10	5
4	Lipophilicity (Log P)	≤4.15	3.05

Result: Pass (0 violations)

N-Nororientaline (Structure shown in figure-2) has been identified as the natural DPP-IV inhibitor. It has been extracted from *Erythrina variegata, Erythrina crystagalli, Erythrina indica, Erythrina poeppigiana*, and *Erythrina arborescens.*[16] The molecular formula and molecular weight of N-Nororientaline are $C_{18}H_{21}NO_4$ and 315.36 g/mol respectively. This molecule follows the Lipinski rule with 0 violations. The aqueous value (Log S) of the molecule is -3.52, identifying it as a soluble molecule. The lipophilicity Log P (octanal-water distribution coefficient) value is 3.05 i.e., it passes the rule. From the structure point of view, it contains 5 H-bonds acceptors and 3 H-bond donors. The molar refractivity of the molecule is 92.11. N-Nororientaline has been predicted to have GI absorption and BBB penetration. It is an active substrate for p-glycoprotein (p-gp). It can inhibit cytochrome p450 (CYP2D6 and CYP3A4). While analyzing the various toxicity aspects of the molecule, it is classified as a class 4 compound (harmful if swallowed). The predicted LD_{50} dose is 700 mg/kg. It has been predicted to be a high immunotoxic and medium risk for hERG inhibition. The molecule has been identified as non-mutagenic.

3.2 Cyanidin 3,5-diglucoside

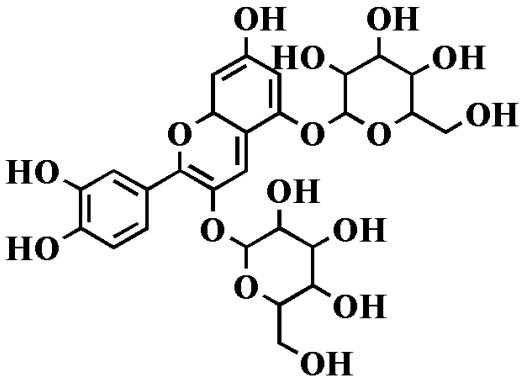

Figure-3- Structure of Cyanidin 3,5-diglucoside

Table: 3 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	611.53 g/mol
2	Number of hydrogen bond donors	≤5	11
3	Number of hydrogen bond acceptors	≤10	16
4	Lipophilicity (Log P)	≤4.15	-5.96

Result: Fail (3 violations)

Cyanidin 3,5-diglucoside (Structure shown in figure-3) has shown 81% DPP-IV inhibition property.[17] This compound has been isolated from *Aronia arbutifolia,* belongs to family Rosaceae.[18] The molecular formula and molecular weight are C27H31O16+ AND 611.53 g/mol. This molecule didn't pass the Lipinski rule as there are 3 violations present. The orally active drug is designed to the molecule that passes the Lipinski rule (not more than 1 violation). The Log S value (aqueous solubility) is determined to be -1.66 indicating its good solubility. Its Log P value (lipophilicity) didn't qualify for the ideal condition that is less than 4.15. Log P value was found to be -5.96.

From the structure point of view, it contains 16 H-bonds acceptors and 11 H-bond donors. This again violated the Lipinski rule. The molar refractivity of the molecule is 140.42. The molecule has been predicted to have low GI absorption and no BBB penetration. It is an active substrate for p-gp. It has predicted to have no effects on enzyme metabolism. The toxicity study has predicted its LD_{50} to 5000mg/kg with 69.26% prediction accuracy. This molecule has been classified as class V molecule on the toxicity scale i.e., may be harmful if swallowed. The molecule is immunotoxicity with 0.60 probability. hERG inhibition by the molecule is ambiguous. There is no carcinogenicity predicted.

3.3 Diprotin A

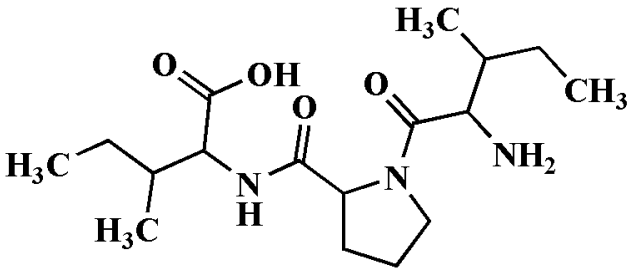

Figure-4- Structure of Diprotin A

Table: 4 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	341.45 g/mol
2	Number of hydrogen bond donors	≤5	3
3	Number of hydrogen bond acceptors	≤10	5
4	Lipophilicity (Log P)	≤4.15	2.14

Result: Pass (0 violations)

Diprotin A (Structure shown in figure-4) (Ile-Pro-Ile) was the first discovered natural DPP-IV inhibitor. It was first isolated from the bacteria.[19] The molecular formula and molecular weight of the molecule are $C_{17}H_{31}N_3O_4$ and 341.45 g/mol respectively. The molecule qualifies the Lipinski rule of 5 with 0 violations. The molar refractivity of the molecule is 96.21. The Log S (aqueous solubility) of the molecule is -0.64 i.e., very soluble. The partition coefficient of the molecule (Log P) qualifies the ideal condition i.e. less than 4.15. Log P value has been predicted to be 2.14. It has high GI absorption and no BBB penetration. It is active substrate for p-gp and do not interfere with enzyme metabolism. From the toxicity study profiling, The LD_{50} dose has been predicted to be 3000 mg/kg with 72.9% prediction accuracy. The LD_{50} dose classifies the molecule to be in class 5 i.e., may be harmful if swallowed. It has low risk of hERG inhibition.

3.4 Amentoflavone

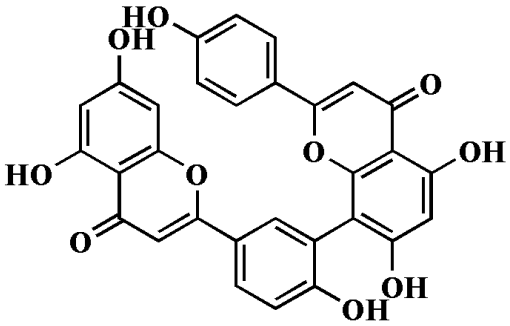

Figure-5- Structure of Amentoflavone

Table: 5 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	538.46 g/mol
2	Number of hydrogen bond donors	≤5	6
3	Number of hydrogen bond acceptors	≤10	10
4	Lipophilicity (Log P)	≤4.15	3.06

Result: Fail (2 violations)

Amentoflavone (Structure shown in figure-5) is extracted from leaves of *Antidesma madagascariense*, family Euphorbiaceae.[20] It has shown to exhibit the DPP-IV inhibitory potential with n IC50 value of 3.9 μM.[17] The molecular formula and the molecular weight of the compound is C13H18O10 and 538.46 g/mol respectively. This molecule does not follow the Lipinski rule of 5 wit 2 violations. From the structure point of view, there are 6 hydrogen bonds donor and 10 hydrogen bond acceptors. The molar refractivity of the molecule is 146.97. It has a poor solubility in aqueous medium. The Log S value has been found to be -6.75. The Lipophilicity of the molecule passes the ideal conditions i.e., less than 4.15. It has been predicted to 3.06. The molecule has low GI absorption. From the toxicity profile, the predicted LD_{50} dose is 3919 mg/kg with 68.07 prediction accuracy. On the toxicity scale, this compound comes under class V (may be harmful if swallowed) as per LD_{50} dose. The toxicity study has predicted the compound to be immunotoxic with 0.51 probability. This molecule has active influence on certain Tox21-Nuclear receptor signalling pathways and Tox-21 stress response pathway. It has been predicted to have medium risk of hERG inhibition.

3.5 Stigmasterol

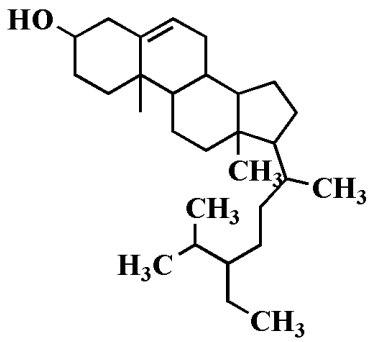

Figure-6- Structure of Stigmasterol

Table: 6 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	412.69 g/mol
2	Number of hydrogen bond donors	≤5	1
3	Number of hydrogen bond acceptors	≤10	1
4	Lipophilicity (Log P)	≤4.15	5.01

Result: Pass (1 violations)

Stigmasterol (Structure shown in figure-6) is extracted from the leaves of *Urena lobata*, family Malvaceae. [21] The study's findings demonstrated that U lobata leaves' ethanolic extract has DPP-IV inhibitory activity, with an IC50 value of 1654.64 g/ml.[17] The molecular formula and the molecule weight of the compound is $C_{29}H_{48}0$ AND 412.69 g/mol respectively. Lipinski rule state that compound should have more than one violation for qualifying as active oral drug. This compound qualifies Lipinski rule with 1 violation. The Log S and Log P values for the compound are -7.46 and 5.01 respectively. The Log S value lower than 0 determines the as compound as poorly soluble in aqueous medium. From the structural bases, it has 1 hydrogen bond donor and acceptor each. In-silico pharmacokinetics study of the compound suggest it has low GI absorption and no BBB penetration. This molecule has predicted to inhibit the cytochrome P_{450} (CYP2C9). This compound is metabolically unstable molecule. The in-silico toxicity study has predicted the LD_{50} doe to be 890 mg/kg. This dose sets this molecule in class IV (harmful if swallowed). The prediction accuracy is 70.97%. It has been predicted to have immunotoxic reactions. There is low risk for hERG inhibition.

3.6 7-deoxy-6-epi-castanospermine

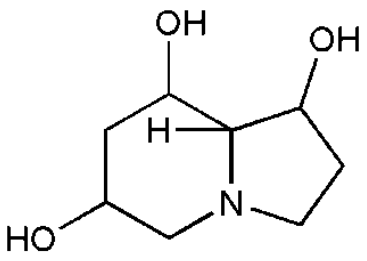

Figure-7- Structure of 7-deoxy-6-epi-castanospermine

Table: 7 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	174.22 g/mol
2	Number of hydrogen bond donors	≤5	4
3	Number of hydrogen bond acceptors	≤10	3
4	Lipophilicity (Log P)	≤4.15	-3.47

Result: Pass (0 violations)

7-deoxy-6-epi-castanospermine (Structure shown in figure-7) is extracted from the immature seeds of the *Castanospermum austral*, family Fabaceae. The ethanolic extract has a high affinity for DPP-IV with an IC_{50} of 13.96 g/ml. This compound has the high affinity for the amino acid residue of the DPP-IV enzyme.[22] The molecular formula and molecular weight of the compound in $C_8H_{16}NO_3$ and 174.22 g/mol. The molecule qualifies the ideal condition to be classified as active oral drug with 0 violation of Lipinski rule of 5. From the structural basis. It has 4 hydrogen bond donors and 3 hydrogen bond acceptors. It has high solubility in aqueous medium with Log S value of -0.13. The Log P value of the compound is -3.47 with qualifies the reference range in accord to Lipinski rule of 5. It has high GI absorption. The compound has been predicted to active substrate of p-gp. According to the toxicity study prediction, LD_{50} dose is 1370mg/kg classifies it as class IV drug (harmful if swallowed). The prediction accuracy is about 70.97%. There is low risk of hERG inhibition.

3.7 Robinin

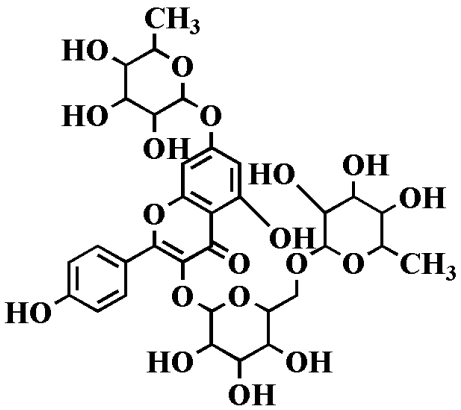

Figure-8- Structure of Robinin

Table: 8 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	740.66 g/mol
2	Number of hydrogen bond donors	≤5	11
3	Number of hydrogen bond acceptors	≤10	19
4	Lipophilicity (Log P)	≤4.15	2.99

Result: Fail (3 violations)

Robinin (Structure shown in figure-8) is isolated from the roots of *Pueraria tuberosa*, family Fabaceae. The extract has demonstrated DPP-IV inhibition with an IC50 value of 17.4 mg/ml.[23] The molecular formula and molecular weight of the compound is $C_{33}H_{40}O_{19}$ and 740.66 g/mol. This compound fails the Lipinski rule of 5 with 3 violations. The log P and log S value of the compound are 2.99 and -3.33 respectively. The log S value of -3.33 makes the molecule to be easily solubilize in aqueous medium. On the structural point of view, the molecule has 11 H-bond donors and 19 H-bond acceptors. The pharmacokinetic studies suggested that it has low GI absorption and no BBB penetration. There is no alteration in the enzyme metabolism by the drug. The LD_{50} dose of the molecule is 5000mg/kg predicted by the in-silico toxicity studies. The drug has the immunotoxic potential with the probability of 0.96. The hERG inhibition ability of the compound is ambiguous.

3.8 Rutin

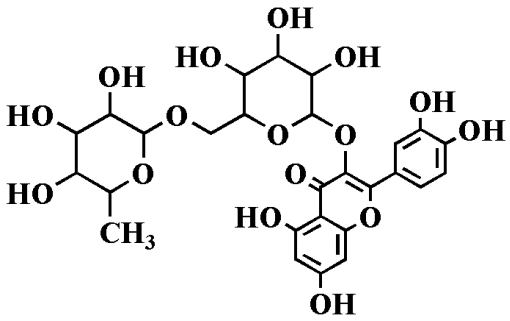

Figure-9- Structure of Rutin

Table: 9 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	610.52 g/mol
2	Number of hydrogen bond donors	≤5	10
3	Number of hydrogen bond acceptors	≤10	16
4	Lipophilicity (Log P)	≤4.15	1.58

Result: Fail (3 violations)

Rutin (Structure shown in figure-9) is obtained from the *Fagopyrum esculentum*, family Polygonaceae, *Ruta graveolens*, family Rutaceae, and *Sophora japonica*, family Fabaceae.[24] The docking study has demonstrated that this compound has the good binding capacity to the DPP-IV enzyme.[25] The molecular formula and molecule weight of the molecule is $C_{27}H_{30}0_{16}$ and 610.52 respectively. The compound doesn't qualify for the Lipinski rule of 5 because its shows 3 violations. The structural analysis shows it has 10 H-bond donors and 15 H-bond acceptors. The molecule is metabolically unstable due to the presence of catechol moiety. The in-silico study has predicted 1 alert in pan assay interference due to catechol moiety i.e., the molecule to give some sort of false positive results in high-throughput screening. The pharmacokinetic studies have suggested its good aqueous solubility.

The log P and log S value of the molecule is 1.58 and -3.30. It has predicted to have low GI absorption and null BBB penetration. The predicted LD_{50} dose is 5000 mg/kg with 100% prediction accuracy. On the toxicity scale, this compound falls under class V (may be harmful if swallowed). The compound has predicted to have immunotoxic nature.

3.9 Antroquinonol

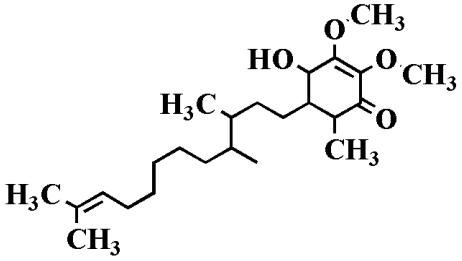

Figure-10- Structure of Antroquinonol

Table: 10 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	390.56 g/mol
2	Number of hydrogen bond donors	≤5	1
3	Number of hydrogen bond acceptors	≤10	4
4	Lipophilicity (Log P)	≤4.15	4.04

Result: Pass (0 violations)

Antroquinonol (Structure shown in figure-10) is extracted form the mycelium of *Antrodia cinnamomea*, family Fomitopsidaceae.[26] The docking study has demonstrated the high docking score of the compound with the DPP-IV enzyme. [25] The molecular formula and molecular weight of the compound is $C_{24}H_{38}O_4$ and 390.56 respectively. The compound follows the Lipinski rule of 5 without any violations. It can be classified as the oral active drug. From the structural analysis, it has 1 H-bond donor and 4 H-bond acceptors. The molecule has been predicted to be metabolically unstable due to presence of the isolated alkene. The molecule didn't follow the leadlikeness rule. The pharmacokinetic studies have predicted the log S and Log P value to be -5.26 and 4.04 respectively. The compound is moderately soluble in the aqueous medium. IT has the high GI absorption and BBB penetration. This compound may influence the enzyme metabolism by inhibiting two cytochrome P_{450} enzymes (CYP2D6 and CYP3A4). It is and active substrate of the p-gp. The toxicity studies predicted the LD_{50} dose 9000 mg/kg. The high LD_{50} makes the compound to be in class VI as non-toxic compound. It has suggested to have low risk of hERG inhibition.

3.10 Curcumin

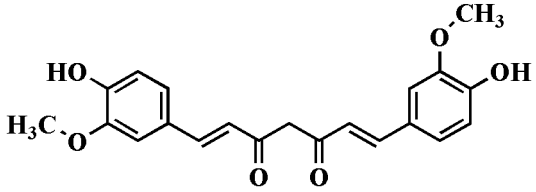

Figure-11- Structure of Curcumin

Table: 11 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	368.38 g/mol
2	Number of hydrogen bond donors	≤5	2
3	Number of hydrogen bond acceptors	≤10	6
4	Lipophilicity (Log P)	≤4.15	3.27

Result: Pass (0 violations)

Curcumin (Structure shown in figure-11) is obtained from the dried rhizome of *Curcuma Longa*, family Zingiberaceae. Curcumin is a strong inhibitor of the DPP-IV enzyme as suggested by the docking and in-vitro studies.[27] The molecular formula and molecular weight of the compound is $C_{21}H_{20}O_6$ and 368.38 g/mol. The molecule qualifies as the active oral drug as it follows the Lipinski rule with 0 violations. On the structural basis the comping consists 2 H-bond donors and 6 H-bond acceptors. Due to the presence of beta-keto anhydride and michael acceptor the compound may be metabolically unstable. Curcumin is easily soluble in aqueous medium; its log S value is -3.94. The predicted lipophilicity of the molecule is 3.27. The pharmacokinetic studies suggested that this compound has good GI absorption but no BBB penetration. It may alter the enzyme metabolism by inhibiting the CYP2C9 and CYP3A4 enzymes. The LD_{50} dose of the compound has been predicted to be 2000 mg/kg. This compound falls under class IV on toxicity scale (harmful id swallowed). The compound has the immunotoxic potential. Curcumin influences the Tox21-stress response pathways such as PPAR-γ, nrf2/ARE, HSE, MMP, and p53 with as probability of 1.

3.11 Calebin A

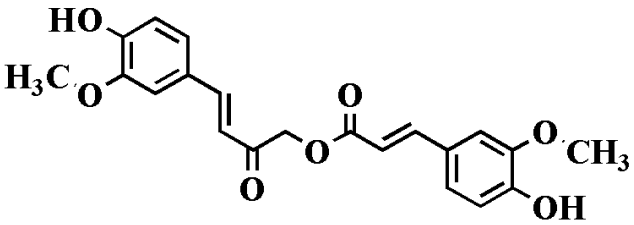

Figure-12- Structure of Calebin-A

Table: 12 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	384.38 g/mol
2	Number of hydrogen bond donors	≤5	2
3	Number of hydrogen bond acceptors	≤10	7
4	Lipophilicity (Log P)	≤4.15	3.33

Result: Pass (0 violations)

Calebin A (Structure shown in figure-12) is obtained from *Curcuma Longa*, family Zingiberaceae. The enzyme inhibition assay has confirmed the good inhibitory action calebin-A on DPP-IV. This compound is structurally related to the curcumin.[28] The molecular formula and molecular weight of the compound is $C_{21}H_{20}O_7$ and 384.38 g/mol. It follows the Lipinski rule with 0 violations. It can be classified as orally active drug. On the structural basis, it consists of 2 H-bond donors and 7 H-bond acceptors. It may be metabolically unstable compound due to presence of Michael acceptor moiety. The pharmacokinetic studies have predicted the log S and log P values to be -4.01 and 3.33 respectively. The compound is moderately soluble in aqueous medium. It can be easily absorbed from the GIT. The drug cannot cross the BBB. IT also influences the enzyme metabolism by inhibiting CYP2C9 and CYP3A4 enzymes. The LD_{50} dose has been predicted to be 978 mg/kg. This drug falls under the class IV on toxicity scale, i.e., harmful if swallowed. The toxicity prediction accuracy is about 68.07%. With the probability of 0.91 it has the immunotoxic potential. This drug also influences the several Tox21-Stressn Response pathways such as nrf2/ARE, HSE, MMP, and p53. The compound has low risk of the hERG inhibition.

3.12 Quercetin

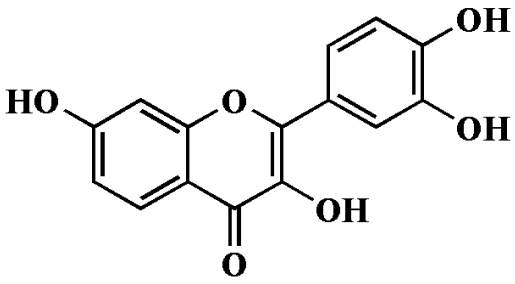

Figure-13- Structure of Quercetin

Table: 13 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	302.24 g/mol
2	Number of hydrogen bond donors	≤5	5
3	Number of hydrogen bond acceptors	≤10	7
4	Lipophilicity (Log P)	≤4.15	1.63

Result: Pass (0 violations)

Quercetin (Structure shown in figure-13) is a bioflavanoid found in approximately 20 plants species such as *Morus alba, Moraceae, Camellia sinensis, Theaceae, Centella asiatica, Apium graveolens, Apiaceae, Coriandrum sativum, Apiceae, Allium cepa, Liliaceae, Asparagus officinalis, Asparagaceae, Prunus domestica, Rosacea,* etc.[29] The docking study and enzyme inhibition assay has suggested quercetin is a strong inhibitor of the DPP-IV enzyme.[30] The molecular formula and molecular weight of the compound is C15H10O7 and 302.24 g/mo.

It is an orally active drug because it follows the Lipinski rule of 5 with 0 violations. The structural analysis reported it has 5 H-bond donors and 7 H-bond acceptors. This compound is metabolically unstable due to presence of the catechol moiety. The pharmacokinetic study predicted the log P and log S value as 1.63 and -3.15 respectively. The molecule has the easily solubility in the aqueous medium. It can be easily absorbed the GIT. This compound also interferes with the enzyme metabolism by inhibiting several cytochromes such as CYP1A2, CYP2D6 and CYP3A4. As per the toxic studies this falls under category III i.e., toxic if swallowed. The LD_{50} dose has been predicted to be 159 mg/kg with 100% prediction accuracy. The compound has both carcinogenic and mutagenic potential with 0.68 and 0.51 probability respectively. The compound has the potential to influence the MMP pathway, ER pathway and ER-LBD pathway. It has the medium risk of the hERG inhibition.

3.13 Puromycin

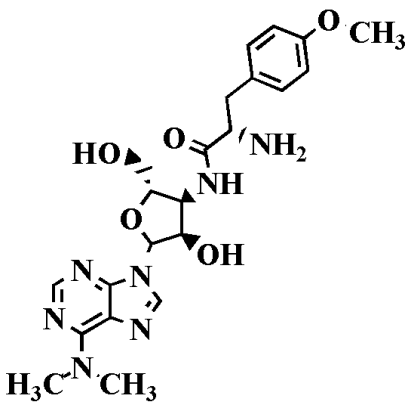

Figure-14- Structure of Puromycin

Table: 14 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	471.51 g/mol
2	Number of hydrogen bond donors	≤5	4
3	Number of hydrogen bond acceptors	≤10	9
4	Lipophilicity (Log P)	≤4.15	1.90

Result: Pass (0 violations)

Puromycin (Structure shown in figure-14) is obtained from a gram positive actinomycete, *Streptomyces alboniger*, family Streptomycetaceae.[31] A docking study has reported that puromycin has the good dock score. IT interacts with amino acid residue Tyr547, Tyr666, Tyr662, Glu206, Arg669 and Phe357 of DPP-IV enzyme.[32] The molecular formula and molecular weight of the compound is $C_{22}H_{29}N_7O_5$ and 471.51 g/mol. The molecule follows the Lipinski rule of 5 with 0 violations. The structural analysis shows presence of 4 H-bond donors and 9 H-bond acceptors. The pharmacokinetic studies have predicted the log S and log P values to be -2.51 and 1.90 respectively. The compound has good solubility in the aqueous phase. It has low GI absorption. The in-silico toxicity studies have predicted the LD_{50} 20 mg/kg. This drug falls under class 2 on the toxicity scale i.e., fatal if swallowed.

It has the high cytotoxic potential and also influence the tumour suppressor p53 gene. The compound represents the high risk of the hERG inhibition.

3.14 16-hydroxycleroda-3,13-dien-15,16-olide (HCD)

3.15

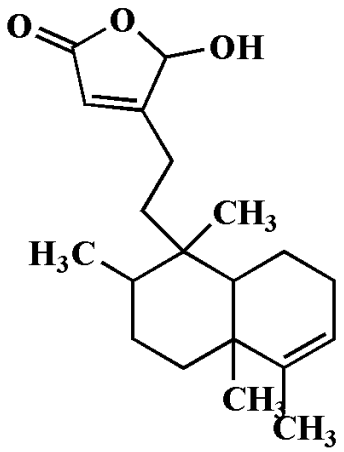

Figure-15- Structure of 16-hydroxycleroda-3,13-dien-15,16-olide (HCD)

Table: 15 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	318.45 g/mol
2	Number of hydrogen bond donors	≤5	1
3	Number of hydrogen bond acceptors	≤10	3
4	Lipophilicity (Log P)	≤4.15	3.10

Result: Pass (0 violation)

16-hydroxycleroda-3,13-dien-15,16-olide (HCD) (Structure shown in figure-15) is a diterpene obtained from the *Polyalthia longifolia*, family Annonaceae.[33] It possesses the antidiabetic property due to the inhibition of the DPP-IV enzyme. The docking has reported a good dockscore.[25] The molecular formula and molecular weight of the compound is C20H30O3 and 318.45 g/mol respectively. This compound qualifies the Lipinski rule of 5 with 0 violations. The structural analysis shows the presence of 1 H-bond donor and 3 H-bond acceptors. The presence of isolated alkene makes the comping metabolically unstable. The predicted log S and Log P values are -4.47 and 3.10 respectively. The molecule is moderately soluble in the aqueous medium. The drug can easily penetrate across the BBB and is have high GI absorption also. It is an inhibitor of CYP2C19 and CYP2C9 cytochrome enzymes. It may orally toxic. The predicted LD_{50} is 34 mg/kg. the drug has been predicted to be in class II i.e., it may fatal if swallowed. The compound represents the medium risk for the hERG inhibition.

3.16 Epigallocatechin gallate

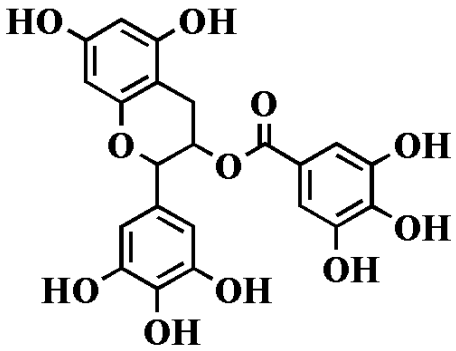

Figure-16- Structure of Epigallocatechin gallate

Table: 16 Lipinski's Rule

S.no	Rule	Ideal Range	Molecule Value
1	Molecular weight	≤500	458.37 g/mol
2	Number of hydrogen bond donors	≤5	8
3	Number of hydrogen bond acceptors	≤10	11
4	Lipophilicity (Log P)	≤4.15	1.87

Result: Fail (2 violations)

Epigallocatechin gallate (Structure shown in figure-15) is natural phenolic compound. It is obtained from the leaves of *Camellia sinensis*, family Theaceae. It has the strong DPP-IV inhibition potential.[34] The molecular formula and molecular weight of the compound is $C_{22}H_{18}O_{11}$ and 458.37 g/mol. It is not an orally active drug because it does not follow the Lipinski rule of 5 with 2 violations. The structural analysis reported it has 8 H-bond donors and 11 H-bond acceptors. This compound is metabolically unstable due to presence of the catechol moiety. The pharmacokinetic study predicted the log P and log S value as 1.87 and -3.56 respectively. The molecule has the easily solubility in the aqueous medium. It has low absorption potential through the GIT. This compound has no interference reported in the enzyme metabolism. As per the toxic studies this falls under category IV i.e., harmful if swallowed. The LD_{50} dose has been predicted to be 1000 mg/kg with 100% prediction accuracy. It represents high risk of the hERG inhibiti

Table 17: *In-silico* ADME studies of 15 secondary metabolites

Sl. No	**COMPOUND**	**LIPINSKI RULE**	**In-Silico ADME studies**							
		PASS/FAIL	**WATER SOLUBILITY**	**GI ABSORPTION**	**BBB PENETRATION**	**P-GLYCOPROTEIN SUBSTRATE**	**CYTOCHROME P450 INHIBITOR**	**BREANS (metabolic stability)**	**PAINS**	**LEADLIKENESS**
1	N-Nororientaline	PASS	SOLUBLE	HIGH	YES	YES	YES (CYP2D6, CYP3A4)	STABLE	0 ALERT	YES
2	Cyanidin 3,5-diglucoside	FAIL	VERY SOLUBLE	LOW	NO	YES	NO	UNSTABLE (CATECHOL, CHARGED OXYGEN AND SUPHUR)	ALERT (CATECHOLA)	NO
3	Diprotin A	PASS	VERY SOLUBLE	HIGH	NO	YES	NO	STABLE	0 **ALERT**	NO
4	Amentoflavone	FAIL	POORLY SOLUBLE	LOW	NO	NO	NO	STABLE	0 ALERT	NO
5	Stigmasterol	PASS	POORLY	LOW	NO	NO	YES (CYP2	UNSTAB	0 ALE	NO

			SOLUBLE				C9)	EL (ISOLATED ALKENE)	RT	
6	7-deoxy-6-epi-castanospermine	PASS	VERY SOLUBLE	HIGH	NO	YES	NO	STABLE	0 ALERT	NO
7	Robinin	FAIL	SOLUBLE	LOW	NO	YES	NO	STABLE	0 ALERT	NO
8	Rutin	FAIL	SOLUBLE	LOW	NO	YES	NO	UNSTABELE (CATECHOL)	ALERT (CATECHOLA)	NO
9	Antroquinonol	PASS	MODERATELY SOLUBLE	HIGH	YES	YES	YES (CYP2D6, CYP3A4)	UNSTABLE (ISOLATED ALKENE)	0 ALERT	NO
10	Curcumin	PASS	SOLUBLE	HIGH	NO	NO	YES (CYP2C9 , CYP3A4)	UNSTABLE (BETA KETO ANHYDRIDE , MICHAEL ACCEPTOR	0 ALERT	NO

11	Calebin A	PASS	MODERATELY SOLUBLE	HIGH	NO	NO	YES (CYP2C9, CYP3A4)	UNSTABLE (MICHAEL ACCEPTOR	0 ALERT	NO
12	Quercetin	PASS	SOLUBLE	HIGH	NO	NO	YES (CYP1A2, CYP2D6, CYP3A4)	UNSTABEL (CATECHOL)	ALERT (CATECHOLA)	YES
13	Puromycin	PASS	SOLUBLE	LOW	NO	YES	NO	STABEL	0 ALERT	NO
14	16-hydroxycleroda-3,13-dien-15,16-olide (HCD)	PASS	MODERATELY SOLUBLE	HIGH	YES	NO	YES (CYP2C19, CYP2C9)	UNSTABLE (ISOLATED ALKENE)	0 ALERT	NO
15	Epigallocatechin gallate	FAIL	SOLUBLE	LOW	NO	NO	NO	UNSTABEL (CATECHOL)	ALERT (CATECHOLA)	NO

Table: 18 *In-silico* toxicity profile of 15 secondary metabolites

S.No	COUMPOUND	In-silico Toxicity studies								
		LD50 DOSE	TOXIXITY CLASS	CARCINOGENIC	MUTAGENIC	IMMUNOTOXIC	CYTOTOXIC	hERG INHIBITION	TOX-21 STRESS RESPONSE PATHWAY	TOX21-NUCLEAR RECEPTOR SIGNALING PATHWAY
1	N-Nororientaline	700 mg/kg	IV	INACTIVE	INACTIVE	ACTIVE	INACTIVE	MEDIUM RISK	INACTIVE	INACTIVE
2	Cyanidin 3,5-diglucoside	5000 mg/kg	V	INACTIVE	INACTIVE	ACTIVE	INACTIVE	AMBIGUOUS	INACTIVE	INACTIVE
3	Diprotin A	3000 mg/kg	V	INACTIVE	INACTIVE	INACTIVE	INACTIVE	LOW RISK	INACTIVE	INACTIVE
4	Amentoflavone	3919 mg/kg	V	INAC	INACTIV	ACTIVE	INACTI	MEDIUM RIS	ACTIVE	ACTIVE

				TIVE	E		VE	K		
									Mitochondrial Membrane Potential (MMP)	Estrogen Receptor Alpha (ER)
									Phosphoprotein (Tumor Supressor) p53	Estrogen Receptor Ligand Binding Domain (ER-LBD)
									Phosphoprotein (Tumor Supressor) p53	
5	Stigmasterol	890 mg/kg	IV	INACTIVE	INACTIVE	ACTIVE	INACTIVE	LOW RISK	INACTIVE	INACTIVE
6	7-deoxy-6-epi-castanospermine	1370 mg/kg	IV	INACTIVE	INACTIVE	INACTIVE	INACTIVE	LOW RISK	INACTIVE	INACTIVE
7	Robinin	5000 mg/kg	V	INACTIVE	INACTIVE	ACTIVE	INACTIVE	AMBIGUOUS	INACTIVE	ACTIVE
										Aryl hydrocarbon Receptor

										(AhR)
8	Rutin	5000 mg/kg	V	INACTIVE	INACTIVE	ACTIVE	INACTIVE	AMBIGUOUS	INACTIVE	INACTIVE
9	Antroquinonol	9000 mg/kg	VI	INACTIVE	INACTIVE	INACTIVE	INACTIVE	LOW RISK	INACTIVE	INACTIVE
10	Curcumin	2000 mg/kg	IV	INACTIVE	INACTIVE	ACTIVE	INACTIVE	MEDIUM RISK	ACTIVE	ACTIVE
									Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)
									Phosphoprotein (Tumor Supressor) p53	Estrogen Receptor Alpha (ER)
									Mitochondrial Membrane Potential (MMP	
									Heat shock factor response element (HSE)	
11	Calebin A	978	IV	IN	INA	ACTI	INA	MEDIU	ACTIVE	INACTIVE

		mg /kg		ACTIVE	CTIVE	VE	CTIVE	MRISK		
									Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	
									Heat shock factor response element (HSE)	
									Mitochondrial Membrane Potential (MMP)	
									Phosphoprotein (Tumor Supressor) p53	
12	Quercetin	159 mg /kg	III	ACTIVE	ACTIVE	INACTIVE	INACTIVE	MEDIUM RISK	ACTIVE	ACTIVE
									Mitochondrial Membrane Potential (MMP)	Aryl hydrocarbon Receptor (AhR)
										Estrogen Receptor Alpha (ER)
										Estrogen Receptor Ligand Binding Domain (ER-LBD)
13	Puromycin	20 mg	II	IN	INA	INAC	AC	HIGH	ACTIVE	INACTIVE

		.kg		ACTIVE	CTIVE	TIVE	TIVE	RISK		
									Phosphoprotein (Tumor Supressor) p53	
14	16-hydroxycleroda-3,13-dien-15,16-olide (HCD)	34 mg/kg	II	INACTIVE	INACTIVE	INACTIVE	INACTIVE	LOW RISK	INACTIVE	INACTIVE
15	Epigallocatechin gallate	1000 mg/kg	IV	INACTIVE	INACTIVE	INACTIVE	INACTIVE	HIGH RISK	INACTIVE	INACTIVE

4. CONCLUSIONS

As per this research, it includes the ADME-T studies of molecules that exhibits DPP-IV inhibitory activity. DPP_IV are classified as the oral hypoglycaemia drugs used for the treatment of T2DM. The in-silico ADME-T studies will help in selecting the best suited natural candidate with good pharmacokinetic profile and less toxicity. Out of the 15 compounds, 10 compounds follow the Lipinski rule of 5. They have been shown to be orally active. The 9 compounds have been predicted to have good water solubility and the GI absorption. As per the research data, antroquinol has shown to be safer drug in terms of toxicity. Further, diprotin A, 7-deoxy-6-epi-castanospermine and 16-hydroxycleroda-3,13-dien-15,16-olide (HCD) also have been shown to be the least toxic among all 15 compounds. N-Nororientaline has shown to posses' good pharmacokinetic profile and Leadlikeness. Diprotin A and 7-deoxy-6-epi-castanospermine have been predicted to the compound with good pharmacokinetic profile with least systemic toxicity. These molecules are metabolically stable. This *in-silico* research data aids in their modification and the creation of useful semi-synthetic molecules.

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