IN-SILICO MOLECULAR DOCKING STUDIES OF HETEROCYCLIC BENZIMIDAZOLE DERIVATIVE AS PROSPECTIVE ANTIDIABETIC AGENTS

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ABSTRACT:

Diabetes affects a large population of the globe and is considered as a leading cause of death. Many synthetic and natural inhibitors have been developed for diabetes treatment. Herein, we report the potential antidiabetic activity of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(4-oxothiazolidin-3-yl)acetamide benzimidazole derivatives, 2-(((1H-benzo[d]imidazol-2-yl)thio)methyl)-5-methyl-1,3,4-oxadiazole) benzimidazole derivatives against the protein namely PDB: 3TOP.

According to the results of the literature review, we can confirm that benzimidazole compounds have higher anti-diabetic activity. Docking was employed for virtual database screening and prediction of the strongest binders based on various score functions. For our present study we used bioinformatics tools such as biological data base PDB (protein data bank), Software like Chem Draw, Chem 3D , Auto PyRx , Discovery studio, Swiss ADME.

Docking experiments on several benzimidazole derivatives were conducted in order to improve antidiabetic efficacy, which is vital for enhancing diabetic chemistry in the public domain. Docking studies of the ligands 1A- 2J were carried out among which 1I, 2G, 1D, 1F displayed promising antidiabetic activity and showed high binding affinity with the target enzyme 3TOP. The Ligands 2D,2C,2I, and 2H, showed highest binding affinity with the values -9.5, -8.9, and -8.8 with rmsd values 0. The ligand such as 1A,1B, 2A, 2B showed high GI Absorption with skin permeation of -6.20 cm/s, -5.96 cm/s, -5.80 cm/s, -5.57 cm/s.

Key words: Anti-diabetic, benzimidazole, Docking study, binding affinity.

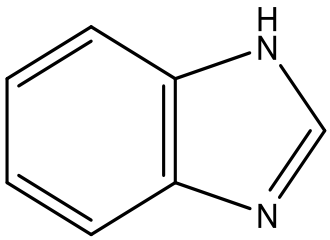
INTRODUCTION:

Diabetes mellitus

Diabetes mellitus is considered one of the main threats to human health in the 21st century. In developing countries, the prevalence of diabetes is increasing, where there are, as estimated by the World Health Organization (WHO), around 70 million people suffering from diabetes mellitus.(1) Changes in human behaviour and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide.(2) Diabetes is a metabolic disorder or a chronic condition where the sugar levels in blood are high. It is also defined as chronic disorders (3)of carbohydrate metabolism due to the lack of insulin result in the hyperglycaemia

and glycosuria. Anyone can be affected by this disease at any age. The type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM) is a multifactorial autoimmune disease[(4)], which susceptibility is determined by a combination of genetic and environmental factors. Diabetes mellitus is one of the most common chronic disorders of childhood.[(5)]

The main cause of diabetes is due to the shortage of insulin or insulin resistance. Glucose is the ultimate source of energy for all metabolic processes. Insulin, a hormone secreted by the pancreas plays a vital role in regulating the movement of glucose and levels of glucose or blood sugar. In the late or advanced stages several complications arise such as related to eyes, kidneys, nerves and blood arteries. As diabetes is a metabolic disorder, people with diabetes are in a risk of other complications associated. The metabolism is affected there by causes various complications. Several changes that occur are due to high rise in blood sugar levels. And hence Diabetes is associated with long-term complications that affect almost every part of the body and often leads to blindness, heart and blood vessel disease, stroke, kidney failure, amputations, and nerve damage. Diabetes is associated with significantly accelerated rates of several debilitating microvascular complications such as nephropathy, retinopathy, and neuropathy, and macrovascular complications such as atherosclerosis and stroke.[(6)]



Benzimidazole

Benzimidazole, a bicyclic hetero-aromatic organic compound consists of the benzene ring and imidazole ring fused at 4- and 5-position.[(7)] Benzimidazoles are named as ortho-phenylene derivatives such as methenyl-o-phenylenediamine and also known as benzoglyoxalines where dimethyl-benzimidazole were first synthesized in 1872 by 2-nitro-4-methylacetanilide.[(7)]Planar benzimidazole ring show tautomerization of H-atom between double-bonded nitrogen atom and single bonded nitrogen atoms to give two chemically equivalent tautomer's that can be written as two sets of numbers to designate the position of the substituents group like 5- methyl benzimidazole or 6-methylbenzimidazole.[(7)] The first benzimidazole was synthesized in 1872 by Hoebrecker via reduction of 2-nitro-4-methyl acetanilide.[(7)]

Molecular Docking

Molecular docking is the most common computational structure-based drug design (SBDD) method and has been widely used ever since the early 1980s [(8)]. It is the tool of choice when the three-dimensional (3D) structure of the protein target is available. Molecular docking popularity has been facilitated by the dramatic growth in availability and power of computers, and the increasing number of and ease of access to small molecule and protein structures. The main goal of molecular docking is to understand and predict molecular recognition, both structurally (i.e., finding possible binding modes) and energetically (i.e., predicting binding

affinity). Molecular docking was originally designed to be performed between a small molecule (ligand) and a target macromolecule (protein) however, in the last decade there has been a growing interest in protein-protein docking, nucleic acid (DNA and RNA)-ligand docking and nucleic acid-protein-ligand docking.[9] The docking can be conducted by application of a number of docking programs, including AutoDock, AutoDock Vina, Molecular Operating Environment (MOE), FlexX, GOLD, and Glide.[10]

Autodock PyRx

AutoDock vina in PyRx software is preferable software in the molecular docking. Its suite of automated docking tools. It is designed to predict how small molecules bind to receptor of known 3D structure. Vina represents shape and properties of the receptor as a grid of points, where each point in space is assigned a value in a field. Advantages of AutoDock vina is that it does not require choosing atom types and pre-calculating grid maps for them.

ADME Studies

Another important parameter we studied was knowing the physicochemical properties, pharmacokinetic parameters, drug likeness, and so on using the bioinformatic tool known as SWISS ADME, which is a free software available for the study of the mentioned parameters.
Swiss ADME gives free access to a number of parameters and predictive models in order to compute the physiochemistry and estimate the pharmacokinetics, drug likeness and medicinal chemistry friendliness of one or several small molecules.
Apart from efficacy and toxicity, many drug development failures are imputable to poor pharmacokinetics and bioavailability. Gastrointestinal absorption and brain access are two pharmacokinetic behaviours crucial to estimate at various stages of the drug discovery processes. To this end, the *Brain or IntestinaL Estimate D permeation* method (BOILED-Egg) is proposed as an accurate predictive model that works by computing the lipophilicity and polarity of small molecules.[11]

MATERIALS AND METHODS:

The different stages involved in molecular docking is as follows:

1. Ligand preparation.
2. Protein preparation.
3. Docking.
4. Evaluation docking results.

There are a range of software packages available for molecular docking like Auto Dock, Hex, Schrodinger GLIDE etc.

For our present study we used bioinformatics tools such as

- Biological data base PDB (protein data bank)
- Software like Chem Draw,
- Chem 3D
- Auto PyRx
- Discovery studio
- Swiss ADME

I. Ligand preparation

- An essential stage in the docking process is ligand preparation. Before we begin the docking process, we must first draw the chemical structure in the Chem draw software. It is an effective tool for creating a wide range of chemical structures. This software not only allows you to draw structures, but it also allows you to name them, among other things.
- After drawing the structures in Chem Draw, they are converted to three-dimensional structures using Chem 3D software.
- Once the structure has been converted into a three-dimensional structure, the following result should be saved in the form of a Protein Data Bank (.pd

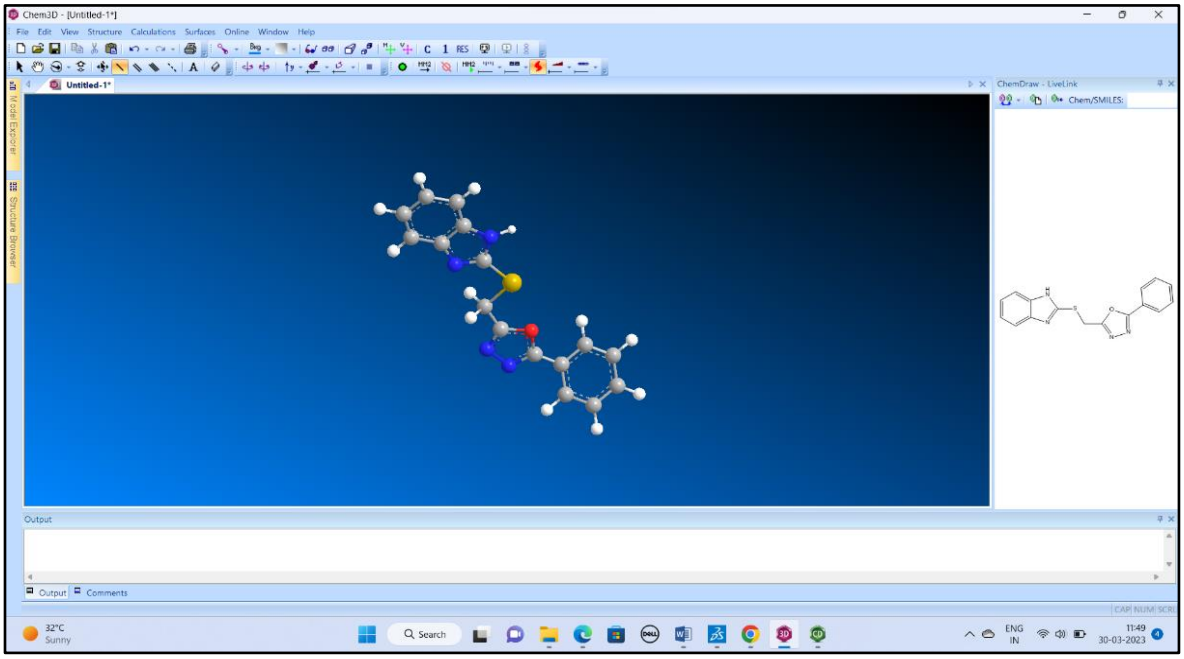


Fig: 1 Showing ligand preparation

II. Protein preparation

Protein preparation is essential in the docking process. A suitable protein must be chosen with which the ligand has the best activity. Several articles were reviewed for our study, and one suitable protein was chosen from among them. The protein selected for our study was 3TOP which is a Human Maltase - Glucoamylase in Complex with Acarbose belonging to the

category of Hydrolase Inhibitor and 2QV4 which is a Human pancreatic alpha-amylase complexed with nitrite and acarbose belonging to the category of Hydrolase Inhibitor.

Following the selection of the protein, we must download the protein from PDB. With the help of PDB one can see the overall properties of the protein. There are lot more information along with that such as knowing the resolution, Ramachandran outliers, viewing of A, B, C and D chain of protein etc.

Once the protein has been downloaded, it should be visualised in the BIOVIA Discovery studio software, where changes such as deleting the unwanted water molecule, chain, or heteroatom can be easily made.

The BIOVIA Discovery Studio Visualizer is a free, feature-rich molecular modelling application for viewing, sharing and analysing protein and small molecule data. Experts and their colleagues can seamlessly and efficiently exchange results, without loss of either time or scientific information.

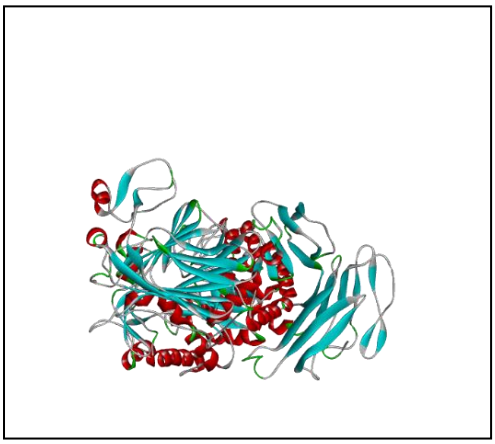


Fig:2 Showing Chain A

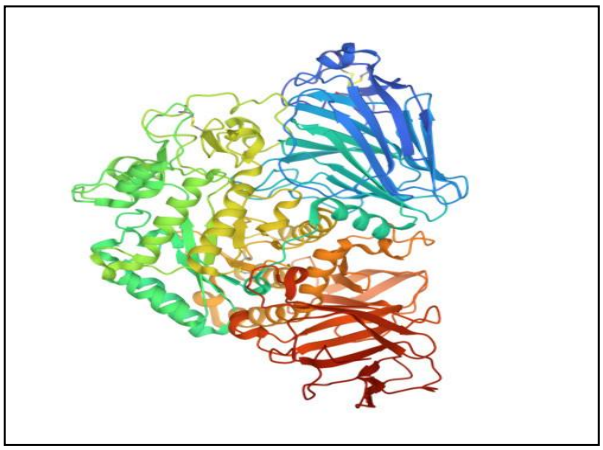


Fig: 3 Showing Chain B

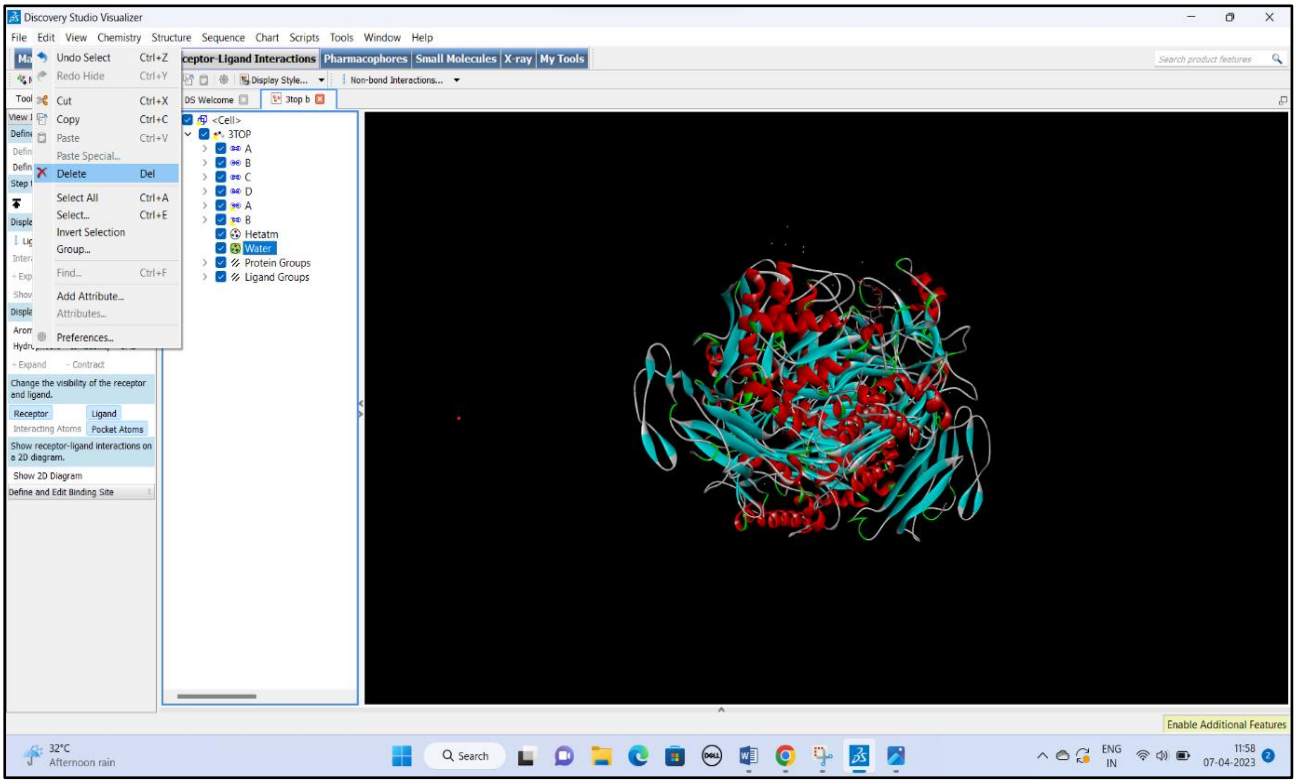


Fig:4 Showing removal of water molecule.

III. Docking

Molecular docking is a kind of bioinformatics modelling which involves the interaction of two or more molecules to give a stable adducts. Docking for our study was done by using Auto dock PyRx which is a free software available.

The docking process consists of several steps. Some of the most important steps are listed below.

1. Before using AutoDock for molecular docking, input files must be converted to the PDBQT file format.
2. The next step is to load the molecule, which involves adding the protein, which is going to be docked with the ligand.
3. The next step is to click the open babel button, which will reveal an option for inserting our ligands.
4. Once we have added all of our ligand molecules, we must minimise the energy.
5. Following this, the ligands must be converted to pdbqt format.
6. After completing these steps, we can begin the docking process, which produces a 2D diagram of the protein binding to the ligand, indicating which ligand binding with amino acid of protein.
7. **Fig:6** shows a clear picture of an amino acid binding with a ligand via bonds such as a hydrogen bond, a pi alkyl bond, and so on.

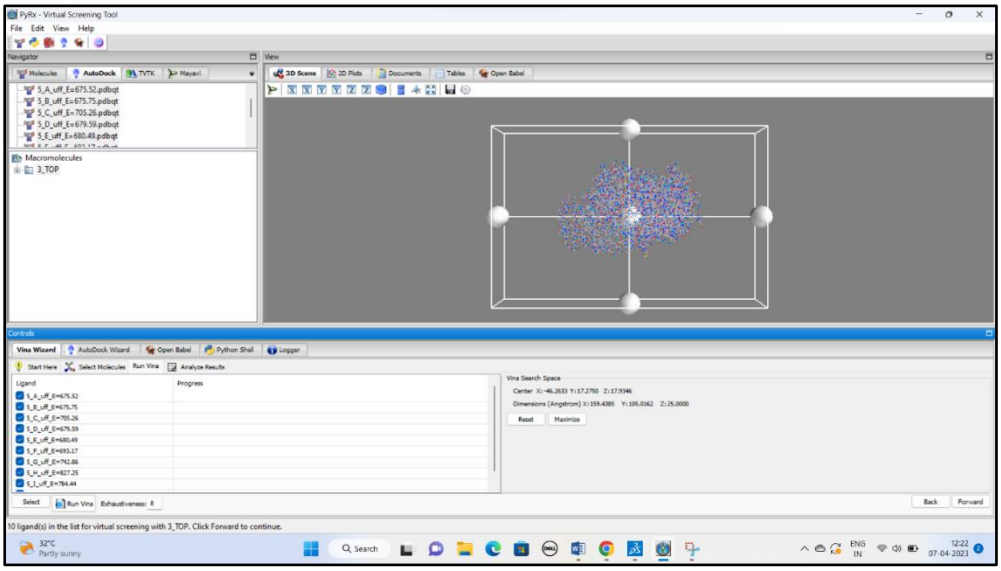


Fig: 5 Showing docking process

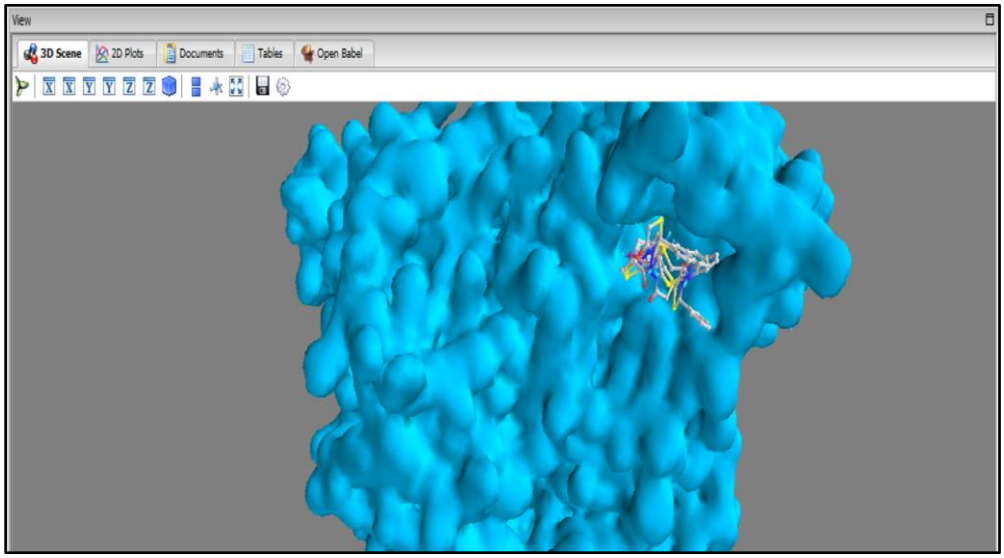


Fig:6 Showing all the ligands binding to protein

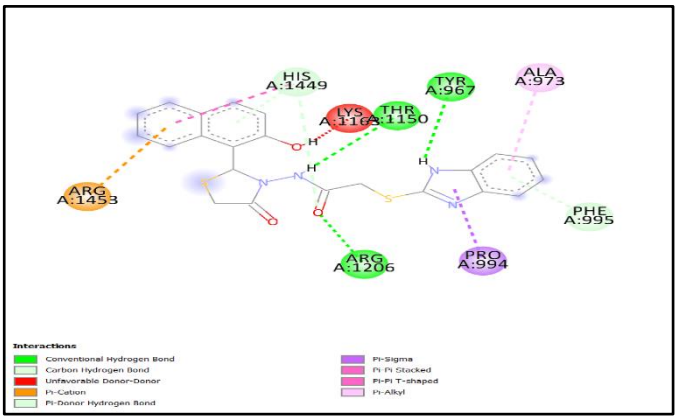


Fig:7 Showing docked 2D image

ADME Studies

Another important parameter we studied was knowing the physicochemical properties, pharmacokinetic parameters, drug likeness, and so on using the bioinformatic tool known as SWISS ADME, which is a free software available for the study of the mentioned parameters.

Steps involved are as follows:

1. Add the structure for which the ADME properties must be determined, as shown in the figure below.
2. After inserting the structure click on >> button and then click on RUN option.
3. This allows us to see the structure's overall properties such as physicochemical, bioavailability, solubility, lipophilicity, and pharmacokinetic parameters.
4. There is a unique model known as BOILED- Egg which is an predictive model that works by computing the lipophilicity and polarity of small molecules.

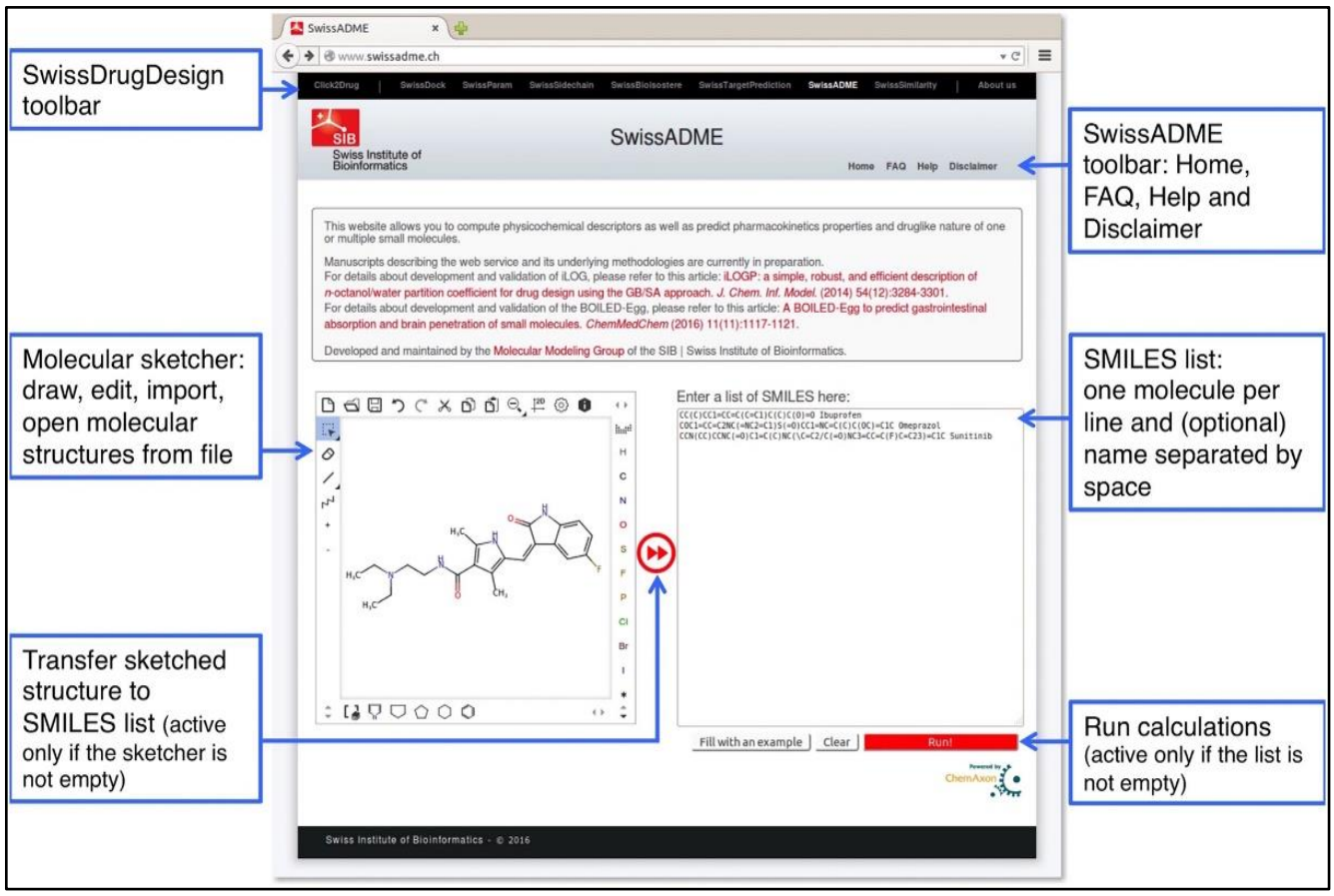


Fig:8 Showing Swiss ADME

RESULTS AND DISCUSSION:

Physicochemical Properties

Physical data of 2-((1H-benzo[d]imidazol-2-yl)thio)-N-(4-oxothiazolidin-3-yl)acetamide benzimidazole derivatives.

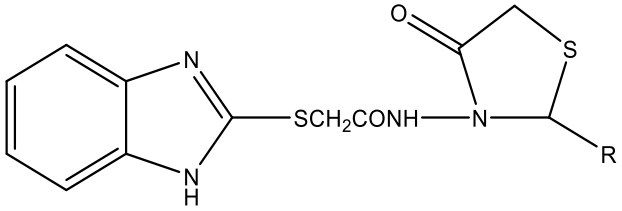
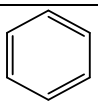
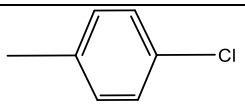
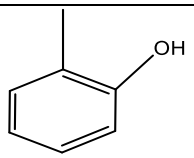
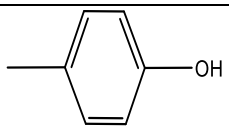
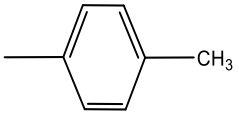
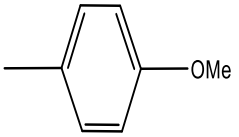
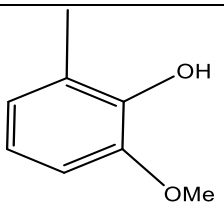
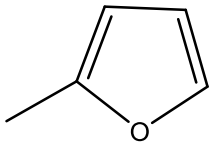
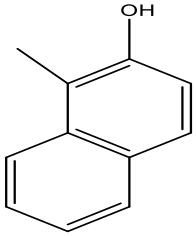
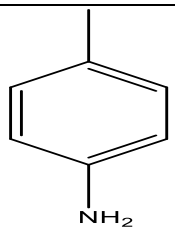


Table No 1: Showing physicochemical data of first set of compounds

Comps.	R	Formula	Molecular weight	TPSA	H-bond acceptor	H-bond donor
1A		$C_{18}H_{16}N_4O_2S_2$	348.48 g/mol	128.69	3	2
1B		$C_{18}H_{15}ClN_4O_2S_2$	418.92 g/mol	128.69	3	2
1C		$C_{18}H_{16}N_4O_3S_2$	400.47 g/mol	148.92	4	3
1D		$C_{18}H_{16}N_4O_3S_2$	400.47 g/mol	148.92	4	3
1E		$C_{19}H_{18}N_4O_2S_2$	398.50 g/mol	128.69	3	2
1F		$C_{19}H_{18}N_4O_3S_2$	414.50 g/mol	137.92	4	2
1G		$C_{19}H_{18}N_4O_4S_2$	430.50 g/mol	158.15	5	3

1H		$C_{16}H_{14}N_4O_3S_2$	374.44 g/mol	141.83	4	2
1I		$C_{22}H_{18}N_4O_3S_2$	450.53 g/mol	148.92	4	3
1J		$C_{18}H_{17}N_5O_2S_2$	399.49 g/mol	154.71	3	3

Physical data of 2-(((1H-benzo[d]imidazol-2-yl)thio)methyl)-5-methyl-1,3,4-oxadiazole) benzimidazole derivatives.

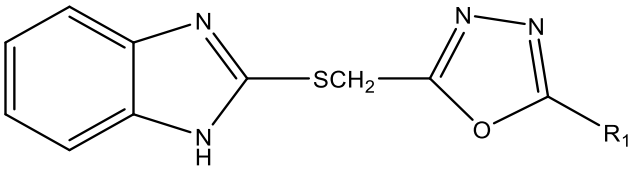
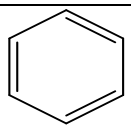
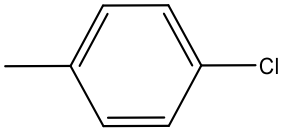
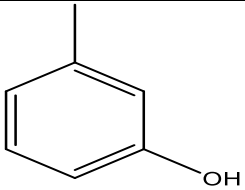
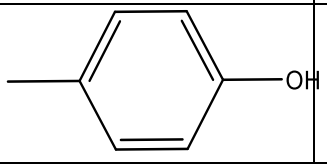
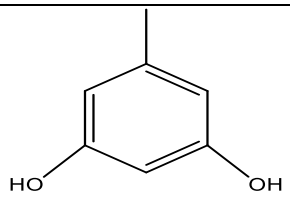
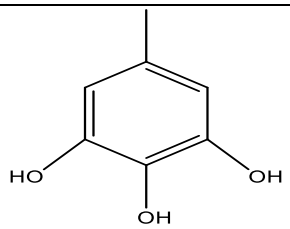
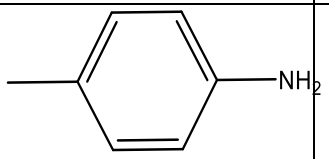
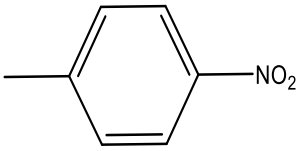
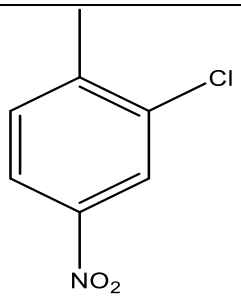
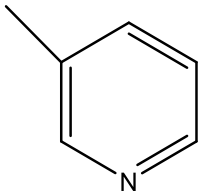


Table No: 2 Showing physicochemical data of second set of compounds

Comp	R	Formula	Molecular weight	TPSA	H-bond acceptors	H-bond donors
2A		$C_{16}H_{12}N_4OS$	308.36 g/mol	92.9	4	1
2B		$C_{16}H_{11}ClN_4OS$	342.80 g/mol	92.9	4	1
2C		$C_{16}H_{12}N_4O_2S$	324.36 g/mol	113.13	5	2
2D		$C_{16}H_{12}N_4O_2S$	324.36 g/mol	113.13	5	2

2E		$C_{16}H_{12}N_4O_3S$	340.36 g/mol	133.36	6	3
2F		$C_{16}H_{12}N_4O_4S$	356.36 g/mol	153.59	7	4
2G		$C_{16}H_{13}N_5OS$	323.37 g/mol	118.92	4	2
2H		$C_{16}H_{11}N_5O_3S$	353.36 g/mol	138.72	6	1
2I		$C_{16}H_{10}ClN_5O_3S$	387.80 g/mol	138.72	6	1
2J		$C_{15}H_{11}N_5OS$	309.35 g/mol	105.79	5	1

Docking result:

The following table shows the binding affinity of the ligand with the protein and also the rmsd value.

Table No: 3 Showing binding energies

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
3_TOP_1_A_uff_E=675.52	-7.5	0	0
3_TOP_1_B_uff_E=675.75	-8	0	0
3_TOP_1_C_uff_E=705.26	-8	0	0
3_TOP_1_D_uff_E=679.59	-7.8	0	0
3_TOP_1_E_uff_E=680.49	-8	0	0
3_TOP_1_F_uff_E=693.17	-7.2	0	0
3_TOP_1_G_uff_E=742.86	-7.7	0	0
3_TOP_1_H_uff_E=827.25	-7.2	0	0
3_TOP_1_I_uff_E=784.44	-8.7	0	0
3_TOP_1_J_uff_E=679.54	-8	0	0
3_TOP_2A_uff_E=645.05	-9	0	0
3_TOP_2_B_uff_E=645.11	-8.4	0	0
3_TOP_2_C_uff_E=651.56	-8.9	0	0
3_TOP_2_D_uff_E=4218.92	-9.5	0	0
3_TOP_2_E_uff_E=655.90	-8.5	0	0
3_TOP_2_F_uff_E=667.15	-8.7	0	0
3_TOP_2_G_uff_E=650.81	-8.2	0	0
3_TOP_2_H_uff_E=677.13	-8.8	0	0
3_TOP_2_I_uff_E=697.15	-8.9	0	0
3_TOP_2_J_uff_E=638.35	-8.6	0	0

Binding affinity is the strength of the binding interaction between a single biomolecule (e.g. protein or DNA) to its ligand/binding partner (e.g. drug or inhibitor).

- The highest binding affinity was demonstrated by the ligand 2D,2C,2I, and 2H, which had values of -9.5, -8.9, and -8.8.

2D Docked Diagram

Following are the images showing the ligands binding to amino acid of the protein.

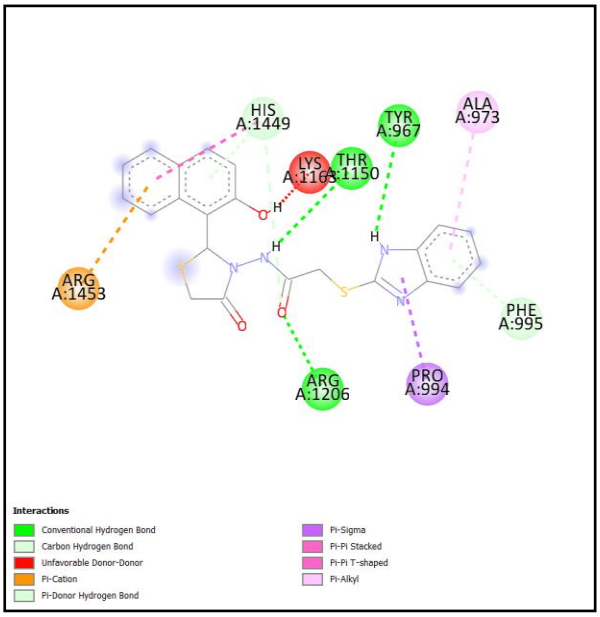


Fig: 9 1 I

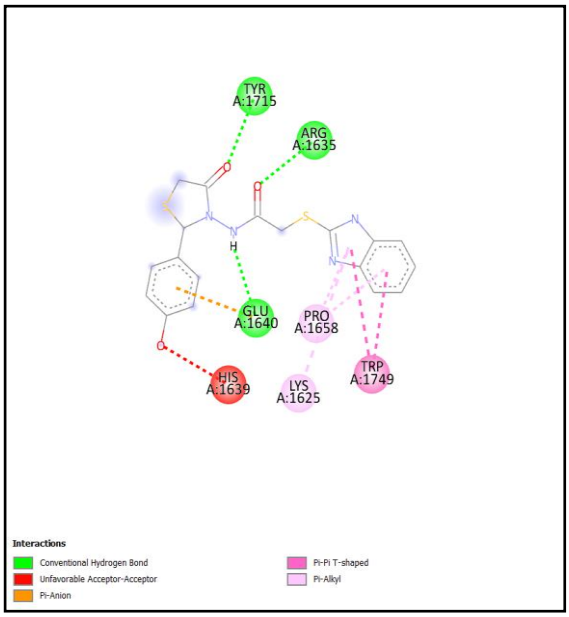


Fig:10 1 D

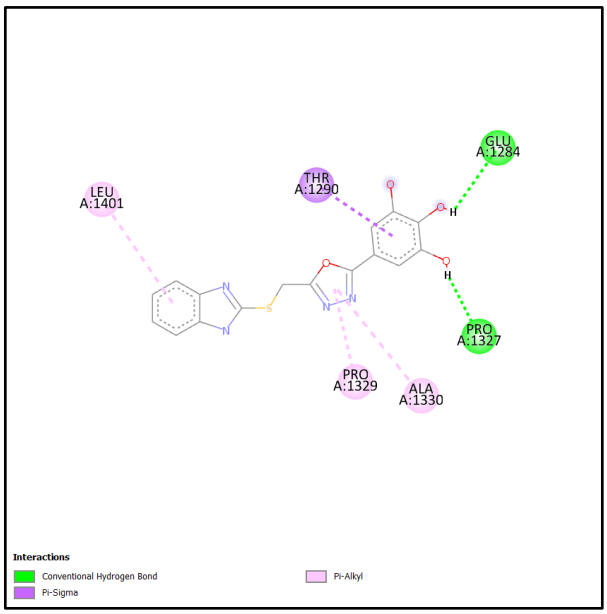


Fig: 11 2 F

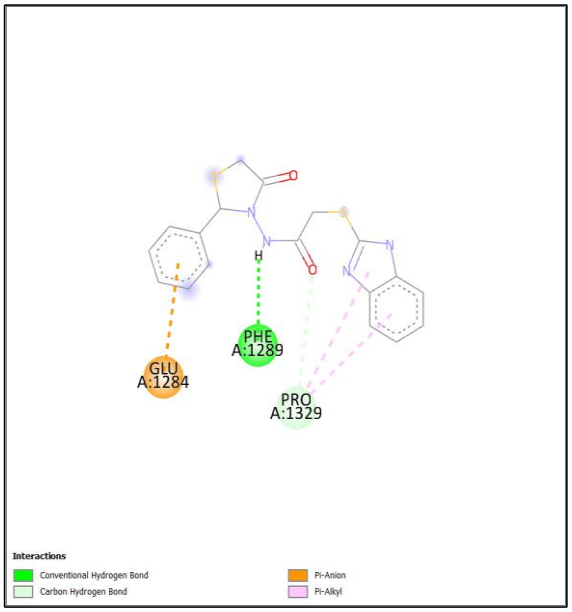


Fig: 12 1 A

3D Docked diagram

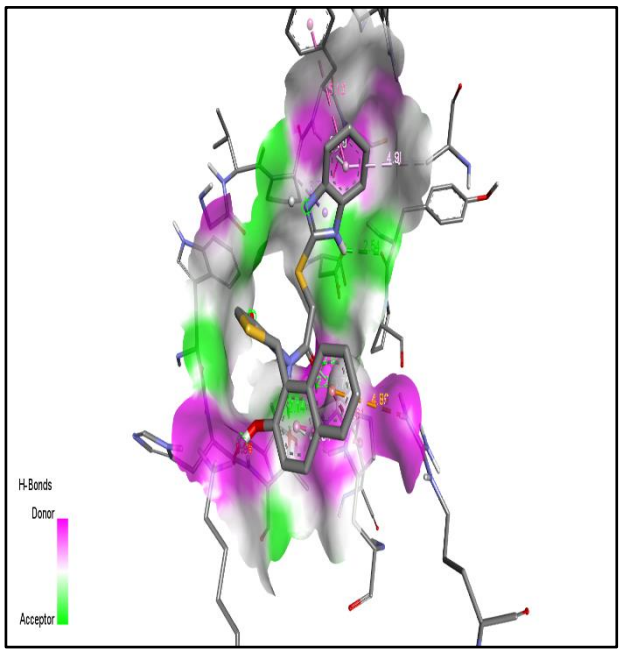


Fig:13 1I

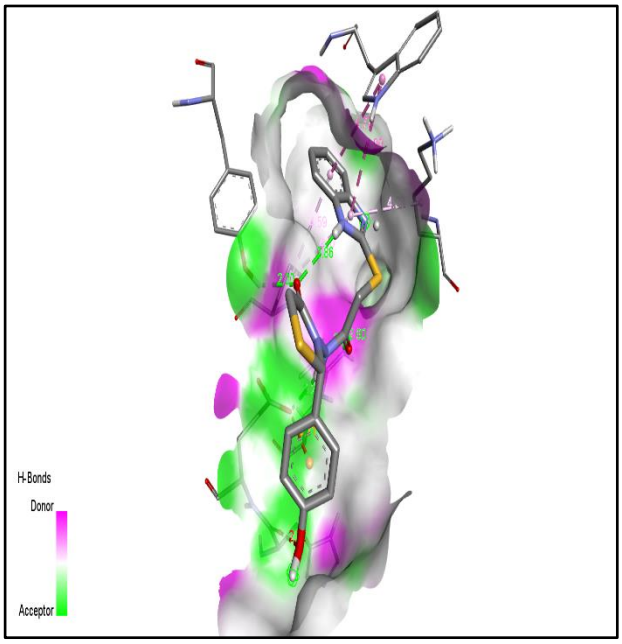


Fig:14 1D

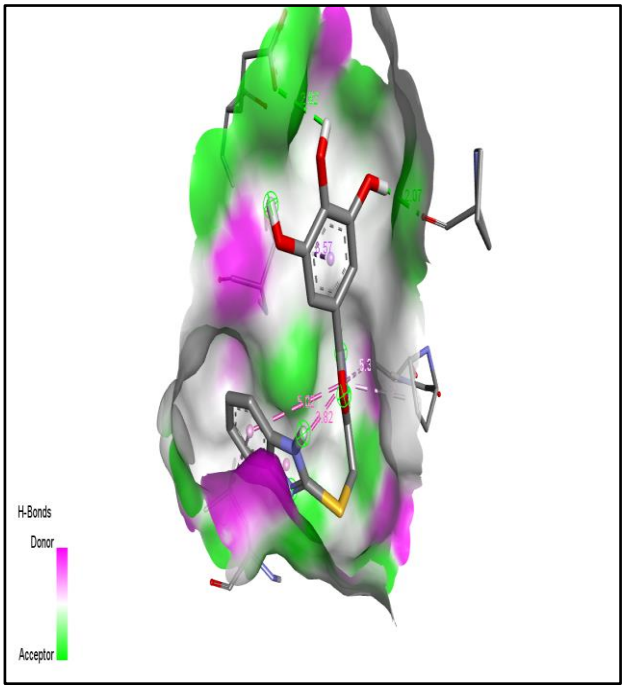


Fig:15 2F

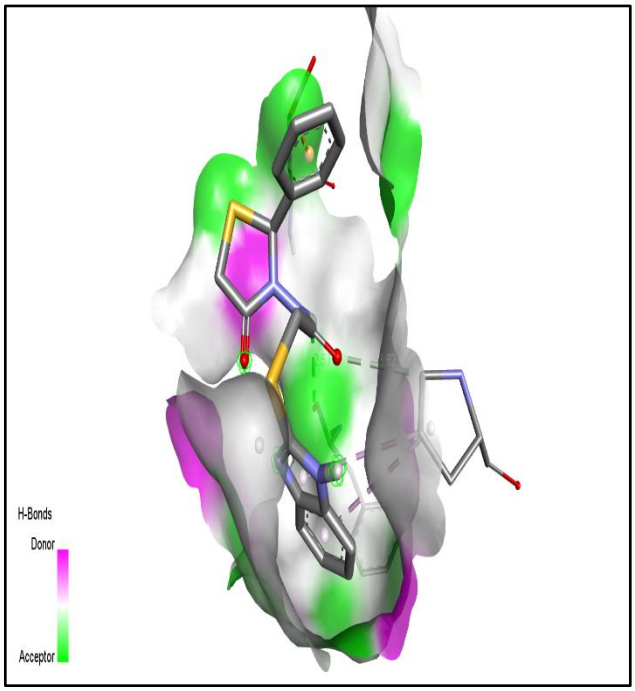


Fig: 16 1A

To investigate the mechanism of anti- diabetic activity and detailed intermolecular interactions, molecular docking studies were performed on the crystal structure of benzimidazole derivatives with (PDB 3 TOP). All the inhibitors along with the ligand were docked. The predicted binding energies of the compounds are listed in table 3. The docking study revealed that all the compounds have showed very good docking score against the enzyme.

As depicted in the **fig: 9**, compound **1I** makes five hydrogen bonding interactions at the active site of the enzyme (PDB 3TOP), among that one interaction were of nitrogen atom with hydrogen atom of **THR 1150** and other interactions were between nitrogen atom with hydrogen atom of **TYR 967**. As depicted in the **fig: 10**, compound **1D** makes three hydrogen bonding interactions at the active site of the enzyme (PDB 3TOP), among that one interaction were of nitrogen atom with hydrogen atom of **GLU 1640**.

As depicted in the **fig: 11**, compound **2F** makes two hydrogen bonding interactions at the active site of the enzyme (PDB 3TOP), among that one interaction were of oxygen atom with hydrogen atom of **PRO 1327** and other interactions were between oxygen atom with hydrogen atom of **GLU 1284**. As depicted in the **fig: 12**, compound **1A** makes one hydrogen bonding interactions at the active site of the enzyme (PDB 3TOP), the interaction was between nitrogen atom and hydrogen atom of **PHE 1289.**

SWISS ADME Result.

Table No:4 Showing ADME properties

	Lipophilicity			**Drug likeness**	
Comps.	$logP_{o/w}$(XLOGP3)	$logP_{o/w}$(WLOGP)	$LogP_{o/w}$(MLOGP)	Lipinski	Veber
1A	3.45	2.26	2.43	Yes; 0 violation	Yes
1B	4.08	2.91	2.93	Yes; 0 violation	Yes
1C	3.09	1.96	1.90	Yes; 0 violation	No;1 violation:TPSA> 140
1D	3.09	1.96	1.90	Yes; 0 violation	No;1 violation:TPSA> 140
1E	3.81	2.56	2.66	Yes; 0 violation	Yes
1F	3.42	2.26	2.13	Yes; 0 violation	Yes
1G	3.06	1.97	1.61	Yes; 0 violation	No;1 violation:TPSA> 140
1H	2.55	1.85	1.19	Yes; 0 violation	No;1 violation:TPSA> 140
1I	4.34	3.11	2.62	Yes; 0 violation	No;1 violation:TPSA> 140
1J	2.77	1.85	1.90	Yes; 0 violation	No;1 violation:TPSA> 140
2A	3.35	3.75	2.72	Yes; 0 violation	Yes
2B	3.98	4.41	3.24	Yes; 0 violation	Yes
2C	3.00	3.46	2.18	Yes; 0 violation	Yes

2D	3.00	3.46	2.18	Yes; 0 violation	Yes
2E	2.64	3.16	1.65	Yes; 0 violation	Yes
2F	2.29	2.87	1.13	Yes; 0 violation	No;1 violation:TPSA> 140
2G	2.67	3.34	2.18	Yes; 0 violation	Yes
2H	3.18	3.66	2.62	Yes; 0 violation	Yes
2I	3.81	4.31	3.13	Yes; 0 violation	Yes
2J	2.28	3.15	1.66	Yes; 0 violation	Yes

- In Drug likeness: Lipinski rule- As depicted in **Table: 4**, all of our ligand molecule follows Lipinski rule as it shows 0 violation.
- Veber rule: As depicted in **Table: 4**, some of the ligands such as 1A, 1B, 1E, 1F follows veber rule and ligands such as 1 C, 1 D, 1G, 1H does not follow Veber rule as it has TPSA value more than 140.

	Pharmacokinetics					
Compounds	GI absorption	BBB permeant	P-gp substrate	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K_p(skin permeation)
1A	High	No	No	Yes	Yes	-6.20 cm/s
1B	High	No	No	Yes	Yes	-5.96 cm/s
1C	Low	No	No	Yes	Yes	-6.55 cm/s
1D	Low	No	Yes	Yes	Yes	-6.55 cm/s
1E	High	No	No	Yes	Yes	-6.03 cm/s
1F	High	No	No	Yes	Yes	-6.40 cm/s
1G	Low	No	Yes	Yes	Yes	-6.75 cm/s
1H	Low	No	Yes	Yes	Yes	-6.77 cm/s
1I	Low	No	Yes	No	Yes	-5.97 cm/s
1J	Low	No	Yes	Yes	Yes	-6.77 cm/s
2A	High	No	Yes	Yes	Yes	-5.80 cm/s
2B	High	No	Yes	Yes	Yes	-5.57 cm/s
2C	High	No	Yes	No	Yes	-6.15 cm/s
2D	High	No	Yes	No	Yes	-6.15 cm/s
2E	High	No	No	No	Yes	-6.50 cm/s
2F	Low	No	No	No	Yes	-6.85 cm/s
2G	High	No	Yes	No	Yes	-6.38 cm/s
2H	Low	No	No	No	Yes	-6.20 cm/s
2I	Low	No	No	No	Yes	-5.96 cm/s
2J	High	No	Yes	Yes	Yes	-6.57 cm/s

- Pharmacokinetics, often known as the activity of pharmaceuticals in the body over time, includes the procedures by which medications are absorbed, transported throughout the body, localised in the tissues, and eliminated.
- In pharmacokinetics: BBB permeation- According to the yolk of the boiled egg all of the ligands show no BBB permeation.
- The ligand such as 1A,1B, 2A, 2B showed high GI Absorption with skin permeation of -6.20 cm/s, -5.96 cm/s, -5.80 cm/s, -5.57 cm/s.

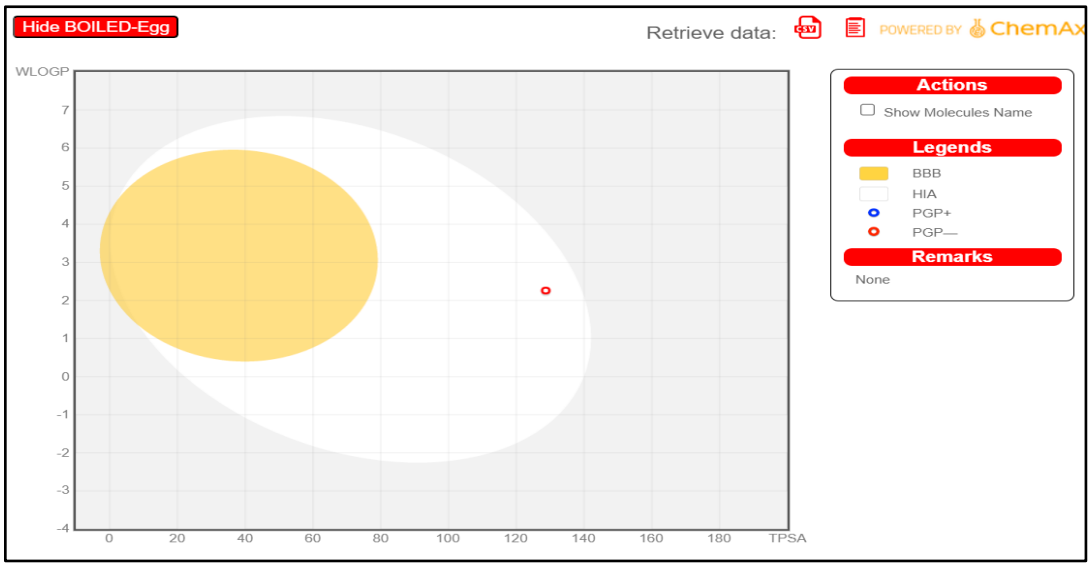


Fig: 17 Showing BOILED-EGG

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