An *in silico* approach to investigate the therapeutic potential of Ayurvedic drugs against Allopathic in treatment of Alzheimer's Disease (Type-3 Diabetes)

A Major Project dissertation submitted

in partial fulfillment of the requirement for the degree of

Master of Technology

In

Biomedical Engineering

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled "**An *in silico* approach to investigate the therapeutic potential of Ayurvedic drugs against Allopathic in treatment of Alzheimer's Disease (Type-3 Diabetes)"** submitted by **Harleen (2K15/BME/03)** in the partial fulfillment of the requirements for the award the degree of Master of Technology (Biomedical Engineering), Delhi Technological University (Formerly Delhi College of Engineering), is a *bona fide* record of the candidate's own work carried out by her under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honouring of any other degree.

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DECLARATION

I, **Harleen** do hereby declare that the dissertation entitled **An *in silico* approach to investigate the therapeutic potential of Ayurvedic drugs against Allopathic in treatment of Alzheimer's Disease (Type-3 Diabetes)** has been undertaken by me for the award of Master of Technology in Biomedical Engineering. I have completed this study under the guidance of **Dr. Pravir Kumar**, Associate professor at Dept. of Biotechnology**,** Delhi Technological University, New Delhi.

I also declare that this dissertation has not been submitted for the award of any Degree, Diploma or any other title in this university or any other university.

Date: 5th July 2017

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LIST OF ABBREVATIONS

Aβ	Amyloid beta
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
BACE-1	Beta Secretase-1
CDK 2	Cyclin-dependent kinases
DM	Diabetes Mellitus
DPP-IV	Dipeptidyl Peptidase IV
eNOS	endothelial Nitric Oxide Synthase
FDA	Food and Drug Administration
GLP-1	Glucagon like peptide-1
GSK 3β	Glycogen synthase kinase 3 beta
HSP 90	Heat Shock Protein 90
NFT's	Neuro Fibrillary Tangles
PDK	Phosphoinositol dependent Kinase
PKA	Protein Kinase A
PPAR	Peroxisome Proliferator activated receptor
PTP1B	Protein-Tyrosine Phosphatase 1B
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
T2DM	Type-2 Diabetes Mellitus
TRPV1	Transient receptor potential cation channel V1
TZD	Thiazolidinedones
VEGF	Vascular endothelial growth factor

An *in silico* approach to investigate the therapeutic potential of Ayurvedic drugs against Allopathic in treatment of Alzheimer's Disease (Type-3 Diabetes)

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1. ABSTRACT

Type-3 Diabetes is a term coined for Alzheimer's disease that is supposed to progress from Type-2 Diabetes Mellitus in response to insulin resistance in brain. It has been reported that more than 5 million people in US are suffering from Alzheimer's Disease and approximately 21 million have Type-2 Diabetes with a continuous increase in number of affected patients. The main symptoms of Alzheimer's disease include cognitive decline, aggression, mood swings, depression, hallucinations, disoriented muscle movement and loss of appetite. Currently, a number of mechanisms have been proposed establishing the crosstalk between both the diseases, the crucial ones being insulin resistance, inflammation and altered insulin signaling. Since, no effective drugs are available in the market till date due to their side effects that slowly worsen the situation after short term of their use, therefore, we have proposed here an alternative treatment strategy using ayurvedic drugs in comparison with the potential drugs that have been or are being used in clinical trials.

Herein, (i) we have identified the target hotspots that have the potential to prevent the progression of both Alzheimer's Disease and Type-2 Diabetes, (ii) predicted the active site residues of the identified targets, (iii) screened the ayurvedic drugs for their drug likeliness and other essential physiochemical properties, (iv) comparatively analyzed the binding affinity and interacting residues of screened targets with ayurvedic drugs with Metformin as a control, (v) identified the putative ayurvedic drug that can be used for ameliorating the symptoms of Alzheimer's Disease and Diabetes.

Keywords: Type-3 Diabetes, Alzheimer's Disease, Type-2 Diabetes, insulin resistance

2. INTRODUCTION

Type-3 Diabetes or Alzheimer's disease (AD) is a neuro-endocrine disorder that proposes insulin resistance as a major cause for the progression of Type-2 Diabetes Mellitus (T2DM) to AD (Mittal *et al.*, 2016). More than 5 million people in US have AD and nearly 21 million people have Diabetes and the number is still increasing. Since, the people who have Diabetes are more prone to developing AD, it can be foreseen that the number of AD will increase up to 4 folds in coming years. So, it becomes a necessity to find a treatment for reducing the symptoms of both AD and DM.

AD is a neurodegenerative disorder that causes memory loss, hallucinations, depression, agitation and dementia with hallmarks of neurofibrillary tangles (NFTs) and amyloid beta plaques (Aβ). T2DM is an endocrine disorder characterized by reduced insulin secretion and insulin resistance. The common risk factor associated with both the diseases is aging. Both diseases share many common features such as cognitive decline, insulin resistance, inflammation and amyloid aggregate formation and mitochondrial dysfunction (Enrique B *et al*., 2014). These cascades of events then lead to progression of Diabetes to AD as summarized in **Figure 1**. The mitochondrial dysfunction causes oxidative stress that leads to generation of ROS (reactive oxygen species) and RNS (reactive nitrogen species). It has been researched that these oxidative species induces accumulation of Aβ plaques in the brain (Misonou H *et al*., 2000). These accumulated Aβ further induces the activation of microglial cells and inflammation in the brain (Cai Z *et al*., 2014). This neuroinflammation further adds to the progression of symptoms of AD.

With the alteration in the insulin signaling pathways, PI3k/Akt signaling gets disturbed which in turn increases the level of GSK 3β. Higher levels of GSK 3β are responsible for hyperphosphorylation of tau protein thus affecting the microtubule organization (Wagner U *et al*., 1996). This microtubular disassembly leads to formation of NFT's. Sometimes, this aggregated Aβ or NFT's starts binding to the neurons thus affecting the transport of various neurotransmitters such as acetylcholinesterase thus causing synaptic loss and neuronal death (Spires-Jones TL *et al*., 2014).

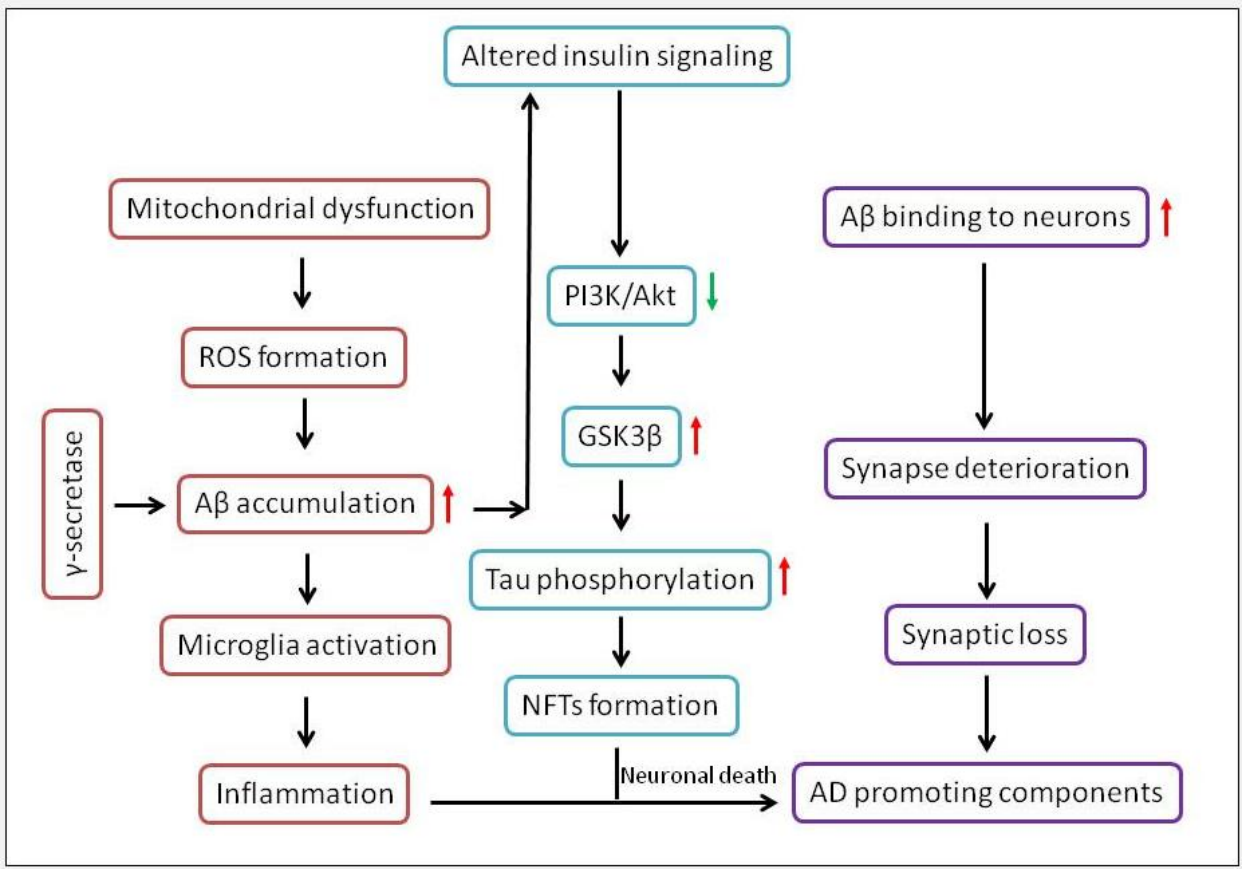

Figure 1: Relationship between AD and altered insulin signaling

In our study, with the help of proposed links, we screened a number of targets and investigated the efficacy of ayurvedic drugs curcumin, capsaicin, sesamol, lupeol and luteolin drugs in comparison to the allopathic drugs that are already in use or are in clinical trials for the treatment of Type-3 Diabetes.

3. REVIEW OF LITERATURE

3.1 IMPLICATIONS FOR GENERATING THERAPEUTICS FOR AD

Since AD and T2DM are linked by insulin resistance and disruption in insulin pathways, there is a possibility that the drugs that are presently used for treatment of T2DM could also help in slowing down the pathogenesis of AD. Here we discuss the different drugs that are used for treatment of T2DM:

3.1.1 ALLOPATHIC DRUGS

3.1.1.1 METFORMIN

Metformin belongs to biguanide class of drugs and is basically used for lowering the blood sugar levels. The levels are reduced by suppressing the hepatic glucose, improving the insulin resistance, reducing fatty acid oxidation and decreasing the amount of glucose absorbed by intestines (Kitabchi *et al.*, 2005). It has also proved beneficial in other diseases such as heart, cancer, PCOS etc (Evans *et al.*, 2005; Currie *et al.*, 2009; Randriamboavonjy *et al.*, 2015). Metformin administered to patients having early stages of cognitive impairment have shown improvements (Domínguez *et al.*, 2012). Since, AD and T2DM are proposed to be linked through insulin resistance; it can prove to be a promising treatment for both the disorders though the mechanisms of action in case of AD is still unknown (Hsu *et al.*, 2011).

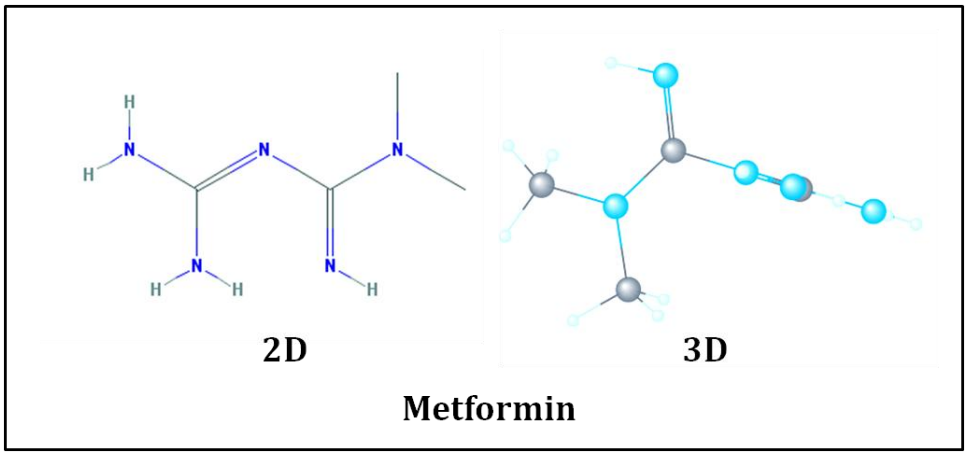

Figure 2: 2D and 3D structures of Metformin

3.1.1.2 DPP-IV INHIBITORS

DPP-IV or Dipeptidyl peptidase IV or 'gliptins' are oral drugs that inhibit the action of DPP-IV. DPP-IV is an enzyme that blocks the action of hormone incretin. Incretin modulates the levels of glucose and insulin produced in the body. The first FDA approved DPP-IV inhibitor is sitagliptin (Herman *et al.*, 2007; Ahren, 2010). Sitagliptin as well as vildagliptin is in clinical trials as it is proposed to significantly delay hallmarks of AD. And also increases the level of GLP-1 (D'Amico *et al.*, 2009). GLP-1 is a hormone that helps in insulin secretion stimulated by glucose and suppresses glucagon secretion. Since, GLP-1 is degraded by DPP; hence DPP-IV inhibitors are used to increase the half life of GLP-1 (Drucker *et al.*, 2006; Deacon, 2011).

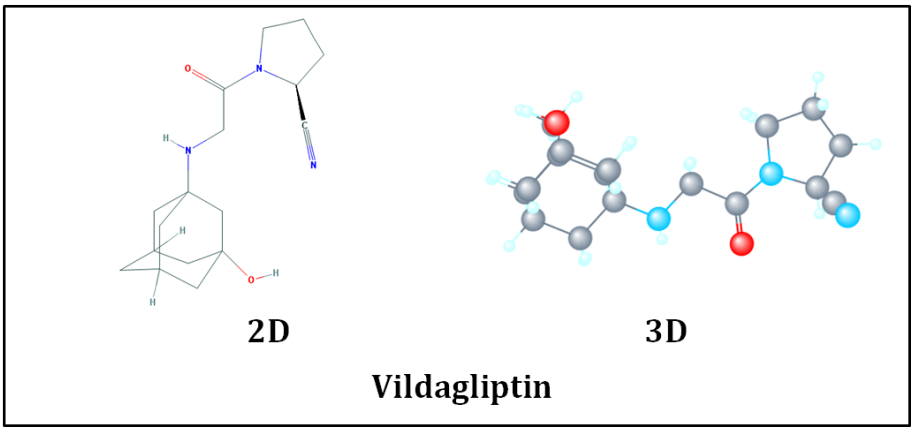

Figure 3: 2D and 3D structures of Vildagliptin

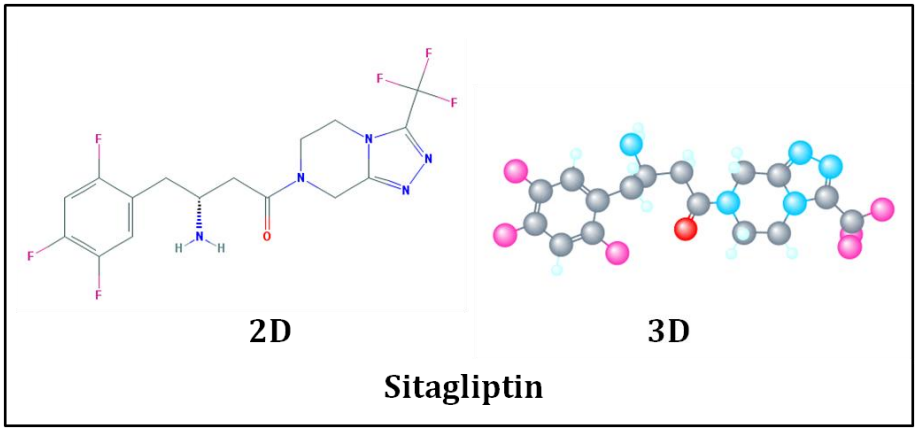

Figure 4: 2D and 3D structures of Sitagliptin

3.1.1.3 THIAZOLIDINEDIONES

Thiazolidinediones(TZDs) or 'glitazones'are a class of drugs that act by binding avidly to PPAR (Peroxisome proliferator-activated receptor) specifically PPARγ. The best PPARγ agonists are pioglitazone and rosiglitazone. They alter the concentration of the hormone adiponectin in order to promote adipogenesis (Greenfield *et al.*, 2004). They also help in decreasing the levels of lipid and fatty acids in liver and muscle and thus act as insulin sensitizers (Yki-Jarvinen, 2004). Since they also have anti-inflammatory properties (Nesto, 2004), these drugs can prove to be a potential treatment for AD (Tuppo *et al.*, 2005; Craft, 2007) and are currently under clinical trials (Watson *et al.*, 2005; Risner *et al.*, 2006; Gold et al., 2010).

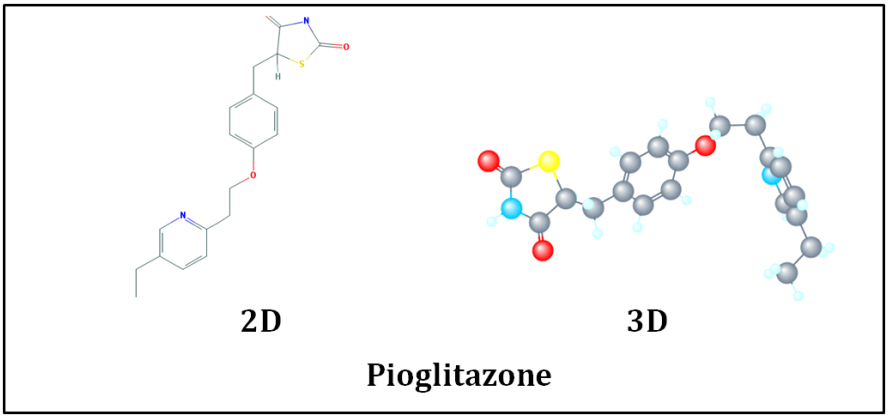

Figure 5: 2D and 3D structures of Pioglitazone

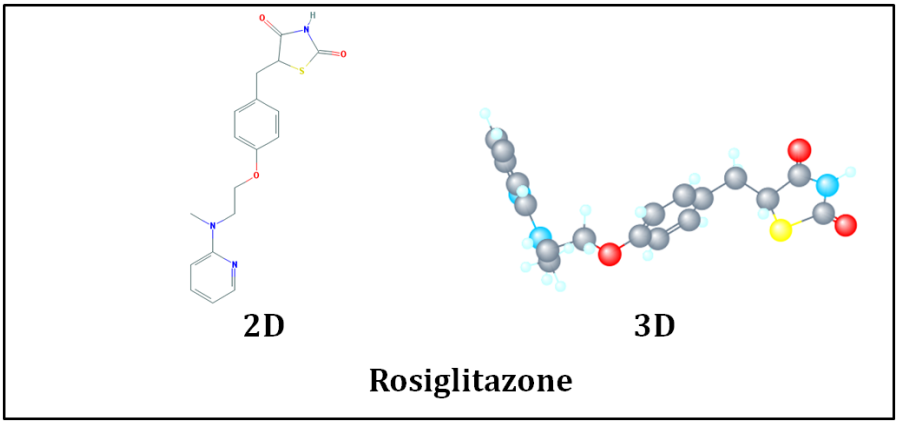

Figure 6: 2D and 3D structures of Rosiglitazone

3.1.2 AYURVEDIC DRUGS

3.1.2.1 CURCUMIN

Curcumin is the principal compound found in turmeric (Curcuma longa Linn.), a famous Indian spice (Kochhar, 2008). Its extract is believed to have a large number of health benefits including anti-diabetic (β cell prevention and insulin resistance) and anti-inflammatory properties (Aggarwal, 2010; Weisberg *et al.*, 2008; Shao *et al.*, 2012; Kuroda *et al.*, 2005; Nishiyama *et al.*, 2005; Jain *et al.*, 2009; Jacob *et al.*, 2007). It is assumed to show anti-diabetic properties by reducing the level of free fatty acids in bloodstream (Seo *et al.*, 2008). Higher amount of free fatty acids can lead to deposition of fat in muscles and liver triggering inflammation and thus disrupting insulin signaling pathways and glucose utilization that can initiate the progression of AD (Jang *et al.*, 2008).

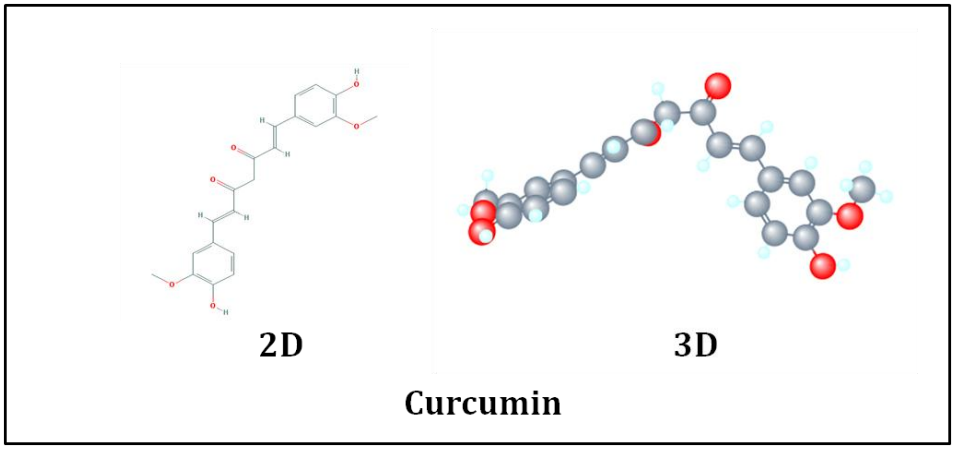

Figure 7: 2D and 3D structures of Curcumin

3.1.2.2 CAPSAICIN

Capsaicin, an active compound found in chilli peppers, is a neuropeptide releasing compound selectively for primary sensory neurons. It is a TRPV1 agonist and shows neuroprotective action but the mechanism is still not known. A research demonstrated that capsaicin helps in activation of TRPV1 that can help in slowing down the hallmarks of AD (Jiang *et al.*, 2013). It is also reported to have anti-inflammatory, anti-oxidant and analgesic properties (Pakaski *et al.*, 2009). It also enhances insulin sensitivity and helps in survival of β-cells by modulating insulin signaling pathways (Kwon *et al.*, 2013). Thus it can prove to be a promising target for therapeutic intervention in AD.

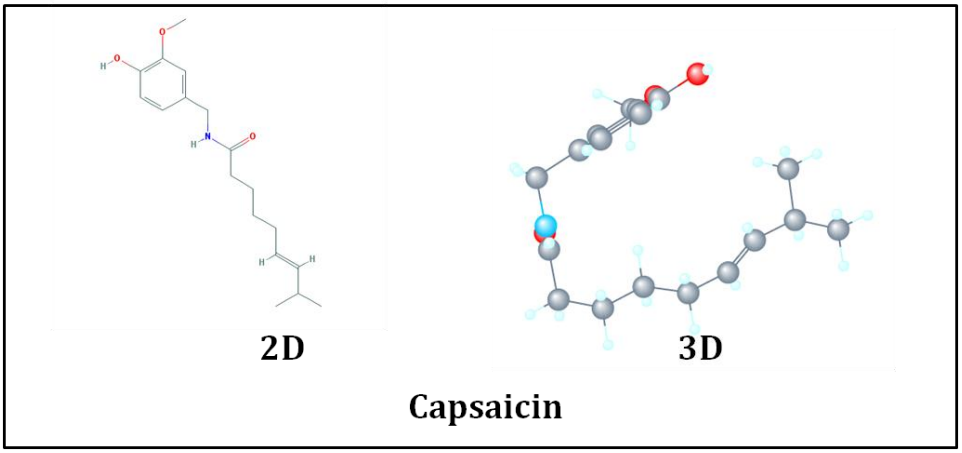

Figure 8: 2D and 3D structures of Capsaicin

3.1.2.3 LUPEOL

Lupeol belongs to a pharmacologically active class known as triterpenoid with molecular formula C30H50O. It can be found in many fruits and vegetables such as mangoes, strawberries, white cabbage, olive, green pepper etc (Wal *et al.*, 2015). It is known to have antimicrobial, anti-inflammatory and anti-tumor properties. In a recent study, it has been depicted that lupeol is involved in the inhibition of various inflammation associated kinases such as MAP Kinase and JNK that are responsible for neuroinflammation and neurodegeneration (Badshah *et al.*, 2016). Also, lupeol and its semi-synthetic derivatives show anti-diabetic properties by increasing the level of anti-oxidant enzymes (Gupta *et al.*, 2012; Lakshmi *et al.*, 2015). Since it has successfully been tested for individual studies of both AD and Diabetes, it can be proposed that lupeol will prove to a good putative target for therapeutic intervention even in combinatorial study of both diseases.

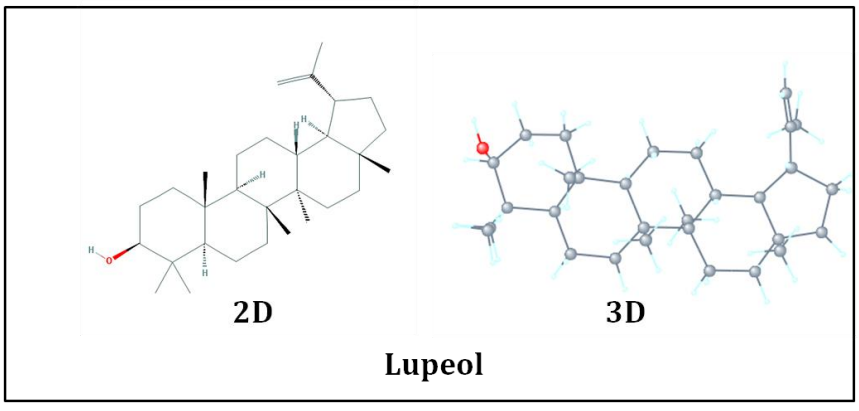

Figure 9: 2D and 3D structures of Lupeol

3.1.2.4 LUTEOLIN

Luteolin, a yellowish crystalline flavonoid often found in chamomile tea, spinach, basil, rosemary, kale, cauliflower, lemons, olive oil, thyme, green peppers, celery. It is widely known for its anti-inflammatory, anti-allegy, anti-cancer, immune and memory booster and antioxidant properties (USDA/Agricultural Research Service ,2010). Its biological effects can be linked to each other. For example, its anti-inflammation effect can be related to its anti-AD properties. Also, it has been proposed that luteolin shows its anti-AD activities by reducing the levels of acetylcholinesterase (AChE) and BACE1 and its anti-diabetic activity by inhibiting PTP1B (Choi *et al.*, 2014; Wang *et al.*, 2016; Kwon, 2017; Zang *et al.*, 2016). Thus it can be used as a potential target for treating both AD and Diabetes Mellitus.

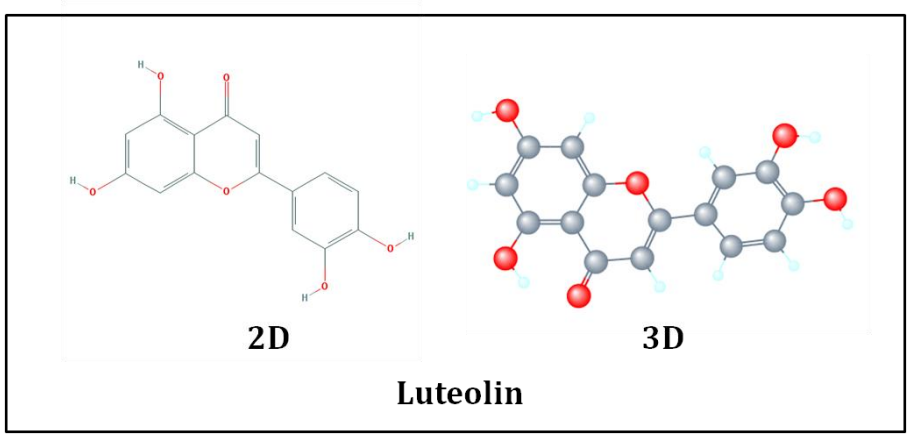

Figure 10: 2D and 3D structures of Luteolin

3.1.2.5 SESAMOL

Sesamol (3,4-methylenedioxyphenol) is a natural white crystalline solid found in sesame oil and sesame seeds. It is known to have anti-oxidant properties; as it can scavenge peroxyl radical and superoxide anion due to its phenolic nature (Hsu *et al.*, 2007; Aboul-Enein et al., 2007; Joshi R *et al.*, 2005; Sonia angeline *et al*., 2013), anti-inflammatory properties; as it reduces the level of TNF-α, which is a marker of inflammation (Kuhad *et al.*, 2008) and anti-fungal properties (Ansari *et al.*, 2014). It has also been investigated that sesamol aids in improving cognitive impairment, inflammation and also increases acetylcholinesterase activity in diabetic rats (Kuhad *et al.*, 2008). But, there is no research that has reported its role in treatment of T2DM. Since it shows reduced inflammation levels, it can be proposed that sesamol can modulate insulin signaling pathways as

well. Thus, it can be concluded that sesamol can show major improvements in inhibiting the disease progression in various neurodegenerative disorders such as AD and also can prove to be ameliorating T2DM at the same time.

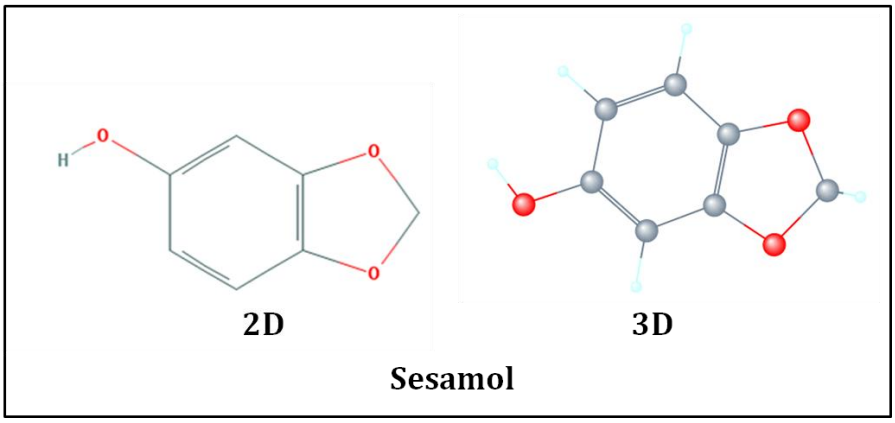

Figure 11: 2D and 3D structures of Sesamol

4. MATERIALS AND METHODS

The methodology used for the study has been described in **Figure 12**.

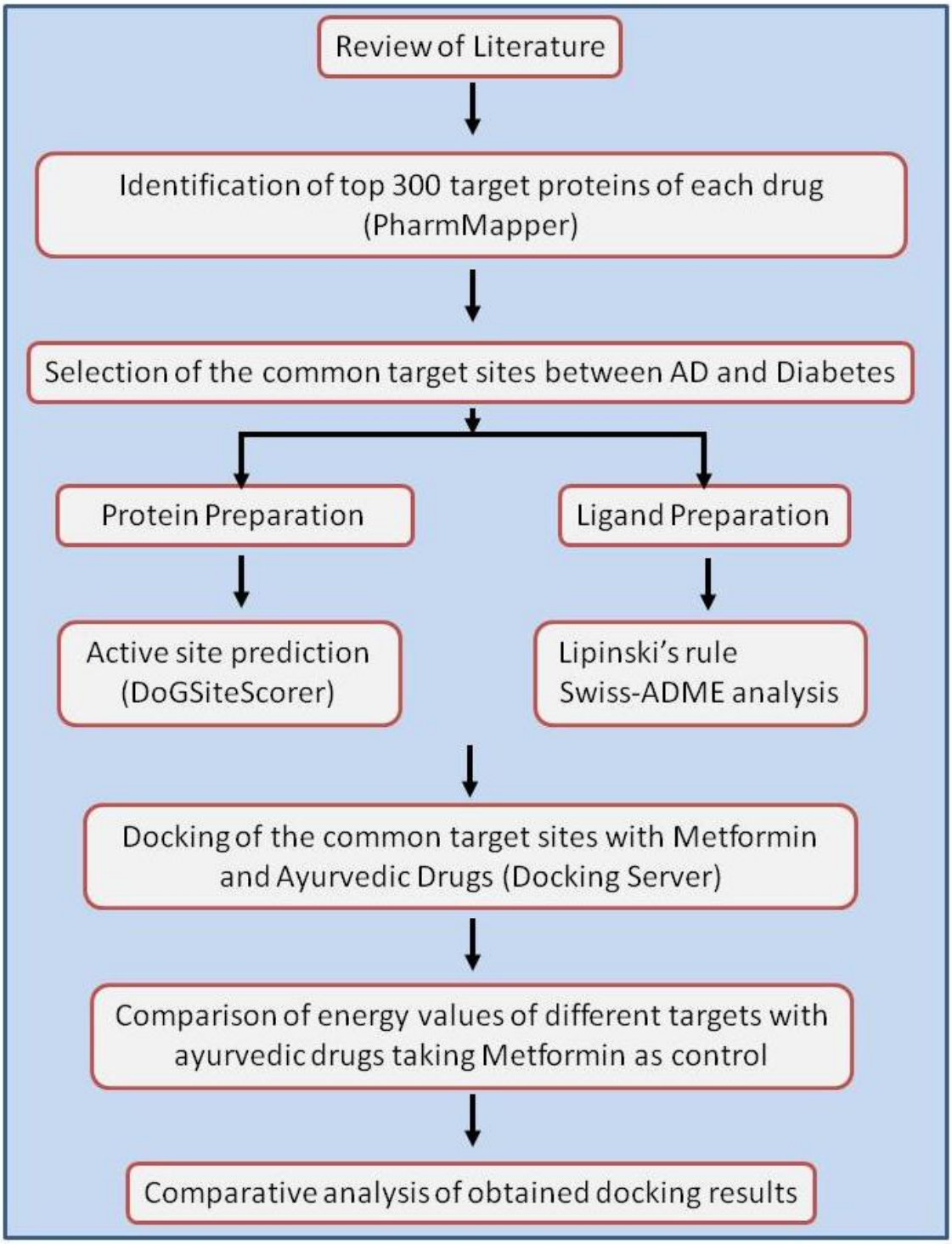

Figure 12: Methodology

4.1 SCREENING OF KEY TARGETS

Potential targets that could link AD and Diabetes were screened through literature survey based on various research articles studying the link, mechanisms and therapeutics of AD and T2DM. The targets were also identified using the bioinformatics tool "PharmMapper" (http://lilab.ecust.edu.cn/pharmmapper/submit_file.php) (Liu *et al.*, 2010; Xia Wang *et al.*, 2016; Wang *et al.*, 2017). It is a web based free online server that uses pharmacophore mapping approach to identify the potential drug targets using the statistical method. All the targets that were identified were further screened to shortlist the common targets linking AD and T2DM on the basis of number of features, fit score and Z'-score. Z'-score is calculated by combining fit score and its subsequent vector together in a score matrix by using pharmacophore method as well as statistical approach. More positive Z'-score value indicates high significance of the target with the drug. The shortlisted targets from the literature survey and from the PharmMapper tool were compiled. A total of twenty targets were listed and further docked with both the ayurvedic and allopathic drugs.

4.2 TARGET LIGAND PREPARATION

The ligand preparation involved the identification of various physiochemical properties and druglikeness parameters of the ayurvedic drugs that needed to be tested. For this, two analyses were done- Lipinski's analysis and Swiss-ADME analysis.

4.2.1 LIPINSKI'S ANALYSIS

Lipinski's rule of five (Pfizer's rule) is used to evaluate the drug likeness of any chemical compound with a biological or pharmacological property. It includes five parameters- mass, hydrogen bond donor, hydrogen bond acceptor, LogP and molar refractivity. All the parameters could be analyzed using the "Lipinski prediction tool" which is freely accessible at http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp (Lipinski, 2004; Jayaram *et al.*, 2012). For a compound to be druggable, it should pass two or more of the five rules stated:

Rule 1: Molecular mass of the drug should be less than 500 Dalton

Rule2: LogP value of the drug should be less than 5

Rule 3: Hydrogen donor bond number should not exceed 5

Rule 4: Hydrogen bond acceptor number should not exceed 10

Rule 5: The value of molar refractivity should lie between 40 and 130

4.2.2 SWISS-ADME ANALYSIS

SWISS-ADME analysis was carried out using the tool "SWISSADME" which is a freely available web server that can be accessed online at http://www.swissadme.ch/ (Daina A *et al*., 2014; Daina, A *et al*., 2017). It is used for the prediction of various parameters such as physiochemical properties, ADME analysis, lipophilicity, water solubility, drug likeness and pharmacokinetics of the chemical compound. Each parameter has multiple sub-parameters that help in depth analysis of the target compound.

4.3 TARGET PROTEIN PREPARATION

The protein preparation involved the identification of active site pocket of the potential target.

4.3.1 ACTIVE SITE PREDICTION

Active site prediction was done to identify the key residues,size, shape and chemical features of the predicted active pocket. It was analyzed using the tool "DoGSiteScorer" (http://proteinsplus.zbh.uni-hamburg.de/pdb_files/search?name=1w50) (Volkamer *et al.*, 2012). It uses the Gaussian filter to classify the potential pockets based on the 3D structure of protein. Higher the drug scores the better the pocket.

4.4 PROTEIN LIGAND DOCKING

Protein-ligand docking was performed using Molecular Docking server tool (https://www.dockingserver.com/web) (Bikadi *et al.*, 2009; Huey *et al.*, 2007). It is a free online tool that is used to perform molecular docking from protein and ligand set-up. It uses a variety of computational softwares for calculations at different stages for more accurate results. The potential ligand that showed the best binding affinity with the target protein was identified based on the maximum negative binding energy.

4.5 COMPARATIVE ANALYSIS

The results from the docking tool were compiled. The comparative analysis was done to conclude an ayurvedic drug that had the maximum binding efficiency to all the identified targets. The

ayurvedic drug could then be suggested as the most effective drug for ameliorating the symptoms of type-2 diabetes and AD.

5. RESULTS

5.1 SCREENING OF KEY TARGETS

The review of literature was done to screen the potential targets for allopathic drugs and ayurvedic drugs that could link both the diseases-AD and Diabetes as shown in **Table 1**. The targets of all the drugs were also identified using the "PharmMapper" tool based on their number of features, fit score and Z'-score values. A collaborative list of 20 screened targets was prepared for both allopathic and ayurvedic drugs. The summarized list for allopathic drugs is shown in **Table 2**.

Table 1: Functions of screened targets

S. No.	Target Protein	Function	Reference
1	VEGF	It significantly prevents Diabetes by restoring the peripheral nerve function and also plays major role in neurodegeneration	(Storkebaum E *et al*., 2004; Del Bo R *et al*., 2009; Religa P *et al*., 2013; Hohman TJ *et al*., 2015)
2	Beta Lactamase	It acts as a inhibitor of Aβ aggregation when combined with other inhibitors	(Lee LL *et al*., 2009)
3	Beta Secretase	It is responsible for the cleavage of Aβ fragment and can also be used as a potential target for treating both AD and Diabetes.	(Vassar R, 2004; Vassar R *et al*., 2014; Shaikh S *et al*., 2016)
4	CDK2	It can be held responsible for changes in tau protein that could lead to AD. Also, its functional loss can lead to Diabetes	(Baumann K *et al*., 1993; Clare PM *et al*., 2001; Kim SY *et al*., 2017)
5	Fructose-1,6-bisphosphatase	It Is helpful in maintaining proper glycemic control	(Van Poelje PD *et al*., 2007; Van Poelje PD *et al*., 2007; Van Poelje PD *et al*., 2011)
6	HSP90	It can be used as a target to control insulin associated neuronal damage specifically AD	(Urban MJ *et al*., 2012; Blair LJ *et al*., 2014)
7	Insulin Receptor	It is a receptor responsible for insulin signalling pathway thus can be used as a potential target in treatment of both AD and Diabetes	(Freude S *et al*., 2009; Bedse, G *et al*., 2015)
8	Neprilysin	It helps in Aβ plaques degradation and can be able a potentail therapeutic target to treat Diabetes	(Marr RA, 2014; Schilling MA, 2016)
9	Thymidylate Synthase	It is a key S phase gene. Over expression of amyloid beta precursor protein binding family B can lead to cell cycle delay that cause downregulation of TS, that is responsible for thymine formation. Decrease in thymine levels can lead to DNA damage & change in gene expression can cause AD	(Bruni P *et al*., 2002; Love JE *et al*., 2015)
10	Phosphotyrosine protein phosphatase	It is a -ve regulator of Insulin pathway. It catalyze dephosphorylation of insulin receptor	(He R *et al*., 2014; Gloria-Bottini F *et al*., 1996)
11	Phosphoinositol dependent kinase (PDK)	It activates Akt pathway which in turn inhibits GSK-3β	(Lee HK *et al*., 2009)
12	Thymidine Kinase	It is a viral gene whose expression is related to the	(Jamieson GA *et al*., 1991)

		incidence of AD	
13	Tyrosine Kinase	Tyrosine kinase Fyn can be a potential therapeutic taregt for ameliorating AD	(Nygaard HB *et al*., 2014; Shirazi SK *et al*., 1993)
14	cAMP dependent protein kinase	It is associated with the NFT's formations in AD	(Davies P *et al*., 1999)
15	PPAR	It is a transcriptional co-activator and has a role in regulation of genes involved in hepatic gluconeogenesis and activates enzymes such as glucose-6-phosphatase and also has a role in AD	(Heneka MT *et al*., 2011; Jay MA *et al*., 2007)
16	eNOS	it is researched to have an associated with AD and DM	(Komolafe A *et al*., 2006; Felaco M *et al*., 2001)
17	Acetylcholinesterase	It has a therapeutic role in lipid metabolism and insulin resistance common to both AD and Diabetes	(Allam AR *et al*., 2006; Felaco M *et al*., 2001)
18	Glutathione S Transferase	It reduces free radical formation. Also, higher levels of this enzyme are related to Diabetic Nephropathy	(Mohini Sharma *et al*., 2016; Tesauro M *et al*., 2015)
19	GSK 3β	Overexpression of GSK 3β induces changes in the brain that can induce hallmarks of AD and Diabetes	(Gao C *et al*., 2011; Hooper C *et al*.,2008; Maixner DW *et al*., 2013)
20	MAP Kinase	It is affects insulin resistance and progressing AD like state	(Drewes G *et al*., 1992; Maixner DW *et al*., 2013; Zhu X *et al*.,2002; Cusi K *et al*.,2000)

Table 2: PharmMapper results of allopathic drugs with the targets

S.No.	Target Name	Parameters	Metformin	Pioglitazone	Rosiglitazone	Sitagliptin	Vildagliptin
1	VEGF	No. of feature	-	8	8	7	8
		Fit score	-	4.04	3.52	3.57	4.47
		Z'-score	-	0.54	-0.83	-0.9	-0.79
2	Beta lactamase	No. of feature	6	-	4	8	7
		Fit score	3.08	-	3.7	3.85	4.5
		Z'-score	1.36	-	0.3	-0.43	-0.84
3	Beta Secretase	No. of feature	13	7	11	8	10
		Fit score	2.96	3.88	3.52	3.57	5.05
		Z'-score	-0.16	-0.16	-1.19	-0.4	2.56
4	CDK 2	No. of feature	7	-	7	7	5
		Fit score	3.04	-	3.56	3.56	4.68
		Z'-score	0.72	-	-0.92	-0.98	0.73
5	Fructose-1,6-bisphosphatase	No. of feature	9	-	10	-	-
		Fit score	2.72	-	3.65	-	-
		Z'-score	-0.89	-	-0.78	-	-
6	HSP 90	No. of feature	4	-	10	8	10
		Fit score	3.04	-	3.5	3.68	4.55
		Z'-score	1.47	-	-1.3	-0.53	-0.71
7	Insulin receptor	No. of feature	-	-	-	-	-

		Fit score	-	-	-	-	-
		Z'-score	-	-	-	-	-
8	Neprilysin	No. of feature	-	10	10	10	-
		Fit score	-	3.93	3.48	3.55	-
		Z'-score	-	-0.81	-1.33	-1.19	-
9	Thymidylate Synthase	No. of feature	10	6	7	8	7
		Fit score	2.81	3.8	3.58	3.61	4.42
		Z'-score	-0.41	-0.76	-0.98	-0.83	-0.68
10	Tyrosine Protein Phosphatase	No. of feature	-	8	7	-	6
		Fit score	-	3.97	3.47	-	5.01
		Z'-score	-	0.21	-1.53	-	2.2
11	Phosphoinositol dependent kinase	No. of feature	-	6	6	7	7
		Fit score	-	3.87	3.87	3.7	4.43
		Z'-score	-	0.17	1.31	-0.76	-1.07
12	Thymidine Kinase	No. of feature	6	6	6	8	-
		Fit score	3.52	3.9	3.6	3.65	-
		Z'-score	2.98	-0.16	-0.57	-0.76	-
13	Tyrosine Kinase	No. of feature	-	7	-	-	-
		Fit score	-	3.85	-	-	-
		Z'-score	-	-0.66	-	-	-
14	cAMP-dependent protein kinase	No. of feature	-	5	5	9	11
		Fit score	-	3.74	3.72	3.56	4.77
		Z'-score	-	-0.13	0.75	-0.17	0.39
15	PGC	No. of feature	-	6	4	6	-
		Fit score	-	3.83	3.82	3.55	-
		Z'-score	-	-0.58	1.34	-0.33	-
16	eNOS	No. of feature	8	6	-	-	-
		Fit score	3.17	3.75	-	-	-
		Z'-score	1.45	-0.76	-	-	-
17	Acetylcholinesterase	No. of feature	-	9	9	8	5
		Fit score	-	5.49	4.84	3.81	4.42
		Z'-score	-	0.95	0.74	0.14	-0.38
18	Glutathione S Transferase	No. of feature	9	8	13	8	11
		Fit score	2.72	3.81	3.66	3.56	4.58
		Z'-score	-0.8	-0.68	-0.9	-0.87	-0.66
19	Glycogen synthase kinase (GSK)	No. of feature	-	-	12	-	-
		Fit score	-	-	3.76	-	-
		Z'-score	-	-	-0.36	-	-
20	MAP Kinase	No. of feature	-	7	-	4	5
		Fit score	-	3.89	-	3.74	4.54
		Z'-score	-	-0.3	-	0.66	0.18

Similarly, the PharmMapper values of all the parameters for similar targets described above have been tabulated in **Table 3** for ayurvedic drugs.

Table 3: PharmMapper results of ayurvedic drugs with the targets

S.No.	Target Name	Parameters	Curcumin	Capsaicin	Lupeol	Luteolin	Sesamol
1	VEGF	No. of feature	-	5	-	-	-
		Fit score	-	3.86	-	-	-
		Z'-score	-	0.22	-	-	-
2	Beta lactamase	No. of feature	4	7	-	7	3
		Fit score	3.86	3.83	-	3.71	2.68
		Z'-score	1.06	-0.26	-	-0.47	0.07
3	Beta Secretase	No. of feature	13	9	13	13	12
		Fit score	3.6	3.98	3.59	3.62	2.64
		Z'-score	-1.13	0.3	-1.04	-1.02	-1.14
4	CDK 2	No. of feature	5	7	6	5	6
		Fit score	3.67	4.34	3.56	3.64	2.82
		Z'-score	-0.22	1.35	-1.09	-0.51	-0.03
5	Fructose-1,6-bisphosphatase	No. of feature	9	9	-	8	6
		Fit score	3.92	4.03	-	3.84	2.87
		Z'-score	-0.52	-0.56	-	-0.76	-0.08
6	HSP 90	No. of feature	-	-	7	8	5
		Fit score	-	-	3.59	3.7	2.7
		Z'-score	-	-	-0.92	-0.49	0.01
7	Insulin receptor	No. of feature	8	-	-	8	-
		Fit score	3.62	-	-	3.77	-
		Z'-score	-1.05	-	-	-0.75	-
8	Neprilysin	No. of feature	-	10	-	10	8
		Fit score	-	3.98	-	3.63	2.79
		Z'-score	-	-0.7	-	-1.13	-0.4
9	Thymidylate Synthase	No. of feature	7	10	8	7	7
		Fit score	3.6	4.87	3.63	3.72	2.84
		Z'-score	-1.1	-0.06	-1.04	-0.57	-0.34
10	Tyrosine Protein Phosphatase	No. of feature	8	8	8	7	10
		Fit score	3.78	3.88	4.23	3.68	3.86
		Z'-score	-0.6	-0.68	-0.03	-0.82	1.31
11	Phosphoinositol dependent kinase	No. of feature	5	7	9	7	-
		Fit score	3.81	4.43	3.6	3.8	-
		Z'-score	0.004	0.4	-0.97	-0.4	-
12	Thymidine Kinase	No. of feature	7	-	-	7	8
		Fit score	3.65	-	-	3.65	2.86

		Z'-score	-0.64	-	-	-0.94	-0.43
13	Tyrosine Kinase	No. of feature	-	7	8	-	12
		Fit score	-	3.78	3.73	-	2.86
		Z'-score	-	-0.91	-0.72	-	-0.69
14	cAMP-dependent protein kinase	No. of feature	6	7	9	8	10
		Fit score	3.69	3.83	3.97	3.88	2.9
		Z'-score	-0.54	-0.12	-0.22	0.27	-0.25
15	PGC	No. of feature	-	-	7	-	-
		Fit score	-	-	3.69	-	-
		Z'-score	-	-	-0.51	-	-
16	eNOS	No. of feature	-	-	6	-	-
		Fit score	-	-	3.65	-	-
		Z'-score	-	-	-0.57	-	-
17	Acetylcholinesterase	No. of feature	-	9	8	9	-
		Fit score	-	4.83	3.74	3.73	-
		Z'-score	-	0.15	-0.74	-0.96	-
18	Glutathione S Transferase	No. of feature	9	11	7	7	7
		Fit score	4.36	4.76	4.4	3.64	2.9
		Z'-score	2.1	1.24	0.58	-0.87	0.1
19	Glycogen synthase kinase (GSK)	No. of feature	-	-	-	7	-
		Fit score	-	-	-	3.86	-
		Z'-score	-	-	-	0.39	-
20	MAP Kinase	No. of feature	4	5	7	10	9
		Fit score	3.63	3.76	3.87	3.74	2.69
		Z'-score	-0.17	-0.27	-0.55	-0.65	-0.54

5.2 TARGET LIGAND PREPARATION

Ligand preparation was done using Lipinski's rule of five and Swiss-ADME analysis.

5.2.1 LIPINSKI'S ANALYSIS

Lipinski's analysis was done to check the drug-likeness of the ayurvedic drugs using the 'Lipinski prediction tool'. The values of all five parameters of Lipinski's rule - mass, hydrogen bond donor, hydrogen bond acceptor, LogP and molar refractivity have been summarized in **Table 4** and the graph comparing all the parameters is shown in **Figure 13**. Since, all the drugs comply with two or more of the five rules, it could be predicted that there is a high probability that they all are drugable molecules.

Table 4: Results of Lipinski's rule of five

Lipinski's Analysis						
S.No.	**Drug Name**	**Mass**	**Hydrogen Bond Donor**	**Hydrogen Bond Acceptor**	**LogP**	**Molar Refractivity**
1	Curcumin	372	0	6	3.344	95.535
2	Capsaicin	301	0	3	3.425	83.127
3	Lupeol	438	1	1	9.292	166.177
4	Luteolin	286	0	6	1.559	62.667
5	Sesamol	215	0	3	2.135	57.119

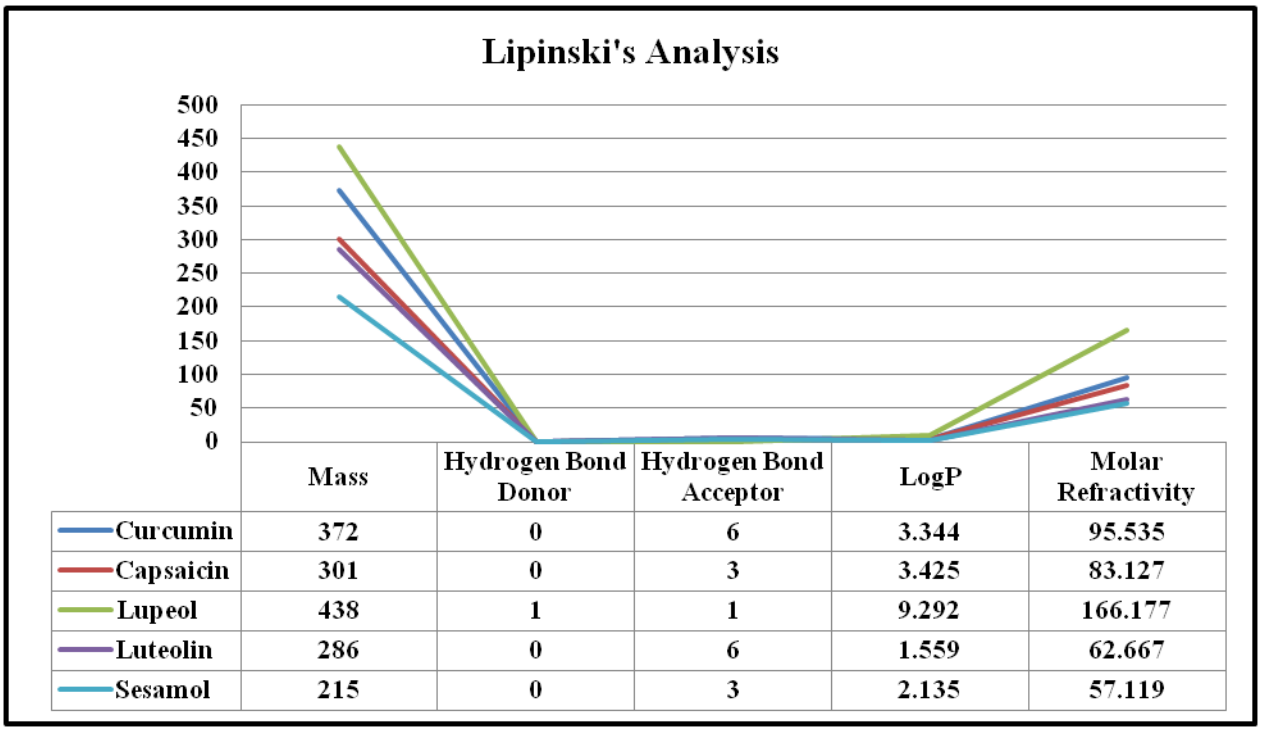

Figure 13: Graph showing results of Lipinski's rule of five

5.2.2 SWISS-ADME ANALYSIS

Swiss-ADME tool was used to evaluate various physiochemical parameters associated with the molecules. The results of this tool are summarized in **Table 5**.

Table 5: SWISS-ADME results

SWISS-ADME Analysis														
S.No.	Drug name	Physiochemical		Lipophilicity		Water Solubility		Pharmacokinetics		Drug Likeness				Synthetic Accessibility
		Formula	Mol. Wt.	iLog P	MLog P	Log S	Class	BBB Permeability	GI absorption	Lipinski	Veber	Ghose	Bioavailability Score	
1	Curcumin	C21H20O6	368.38	3.27	1.47	-3.94	Soluble	No	High	Yes	Yes	Yes	0.55	2.97
2	Capsaicin	C18H27NO3	305.41	3.15	2.69	-3.53	Soluble	Yes	High	Yes	Yes	Yes	0.55	2.32
3	Lupeol	C30H50O	426.72	4.71	6..92	-8.64	Poorly soluble	No	Low	Yes	Yes	No	0.55	5.49
4	Luteolin	C15H10O6	286.24	1.86	-0.03	-3.71	Soluble	No	High	Yes	Yes	Yes	0.55	3.02
5	Sesamol	C10H14O3Si	210.3	3.01	1.50	-3.3	Soluble	Yes	High	Yes	Yes	Yes	0.55	3.3

5.3 TARGET PROTEIN PREPARATION

5.3.1 ACTIVE SITE PREDICTION

Active site prediction was done by DoGSiteScorer tool. The prediction sites for all the targets have been summarized in **Table 6** along with the volume, surface area, drug score and simple score values. The pockets were selected on the basis of the drug score. The higher the value of drug score, the better is the coverage of ligand.

Table 6: Predicted active site residues of all targets along with drug score

SNo	Target Protein	PDB ID	Predicted Active Site Residues	Volume ($Å^3$)	Surface ($Å^2$)	Drug Score	Simple Score
1	VEGF	1VPF	ARG,ASN,ASP,CYS,GLU,GLY,ILE,LEU ,LYS,PHE,PRO,SER,THR,TYR,VAL	1355.17	1728.99	0.8	0.65
2	β- Lactamase	1I2S	ALA,ARG,ASN,ASP,GLN,GLU,GLY,IL E,LEU,LYS, PRO,SER,THR,TRP,VAL	1760.81	2016.23	0.78	0.61
3	β-Secretase	1W50	ALA,ARG,ASN,ASP,GLN,GLU, GLY,ILE,LEU,PHE, PRO,SER,THR,TRP,TYR	261.12	282.09	0.65	0
4	CDK2	1W98	ALA,ARG,ASN,ASP,GLN,GLU,GLY,HI S,ILE,LEU,LYS,MET,PHE,PRO,SER,TH R,TRP,TYR,VAL	1458.94	1408.3	0.81	0.58
5	Fructose-1,6-bisphosphatase	1NUX	ALA,ARG,ASP,GLY,ILE,LEU,LYS,MET ,PRO,SER,THR	229.7	391.53	0.36	0.11
6	HSP90	3Q6M	ALA,ARG,ASN,CYS,GLN,GLU,GLY,HI S,ILE,LEU,LYS,PHE,PRO,SER,THR,TR P,TYR,VAL	1896.12	2267.37	0.8	0.57
7	Insulin Receptor	2HR7	ARG,ASN,CYS,GLN,GLY,HIS,ILE,LEU, LYS,MET,PRO,SER,THR,TRP,TYR,VA L	421.82	535.62	0.57	0.24

8	Neprilysin	5JMY	ALA,ARG,ASN,ASP,GLN,GLU,GLY,ILE,LEU,LYS,PHE,PRO,SER,THR,TRP,TYR,VAL	2136.49	2472.12	0.8	0.59
9	Thymidylate Synthase	1HZW	ALA,ARG,ASN,ASP,CYS,GLN,GLU,GLY,HIS,ILE,LEU,LYS,MET,PHE,PRO,SER,THR,TRP,TYR,VAL	1054.82	1200.91	0.8	0.63
10	Phosphotyrosine protein phosphatase	1PHR	ALA,ARG,ASN,GLU,GLY,HIS,ILE,LYS,PRO,THR	179.01	419.27	0.43	0
11	Phosphoinositol dependent kinase (PDK)	1H1W	ASP,GLU,GLY,ILE,LEU,LYS,PHE,SER,THR,TYR,VAL	306.24	382.79	0.45	0.17
12	Thymidine Kinase	1XBT	ALA,ARG,ASP,GLN,GLU,GLY,ILE,LEU,LYS,MET,PHE,PRO,SER,THR,TYR,VAL	878.41	941.34	0.83	0.51
13	Tyrosine Kinase	1SM2	ALA,ARG,ASN,ASP,CYS,GLN,GLU,GLY,HIS,ILE,LEU,LYS,MET,PHE,SER,THR,VAL	1232.89	1402.13	0.79	0.66
14	cAMP dependent protein kinase	4WIH	ALA,ARG,ASN,ASP,GLN,GLU,GLY,HIS,ILE,LEU,LYS,MET,PHE,SER,THR,TYR,VAL	1072.13	1028.47	0.78	0.56
15	PPAR	2Q8S	ALA,ARG,ASN,ASP,CYS,GLN,GLU,GLY,HIS,ILE,LEU,LYS,MET,PHE,PRO,SER,THR,TYR,VAL	1894.11	2085.01	0.81	0.65
16	eNOS	3NOS	ALA,ARG,ASN,ASP,CYS,GLN,GLU,GLY,ILE,LEU,MET,PHE,PRO,SER,THR,TRP,TYR,VAL	953.41	1025.8	0.82	0.55
17	Acetylcholinesterase	1QTI	ALA,ARG,ASN,ASP,GLN,GLU,GLY,HIS,ILE,LEU,PHE,PRO,SER,TRP,TYR,VAL	850.4	746.5	0.83	0.55
18	Glutathione S Transferase	1R5A	ALA,HIS,ILE,LEU,MET,PHE,PRO,SER,TYR	370.37	566.18	0.62	0.37
19	GSK 3β	1I09	ALA,ARG,ASN,ASP,CYS,GLU,GLY,ILE,LEU,LYS,MET,PHE,PRO,SER,TYR,VAL	1197.18	1302.12	0.81	0.65
20	MAP Kinase	3HVC	ALA,ARG,ASP,GLU,GLY,HIS,ILE,LEU,LYS,MET,PHE,PRO,SER,THR,VAL	833.86	970.27	0.85	0.53

5.4 PROTEIN LIGAND DOCKING

All the targets were docked using Molecular Docking server tool and the energy values generated after docking are summarized in **Table 7**. Here, Metformin was taken as the control. The drug with the minimum estimated free energy of binding has been highlighted in the table in green. Since, some targets such as acetylcholinesterase, Glutathione S transferase, GSK 3β and MAP Kinase had

shown zero or positive energies of binding, these targets had been eliminated from the further analysis. Moreover, their docking results and HB plots have been shown in **Figure 14-29**.

Table 7: Docking results of all the targets with metformin (control) and ayurvedic compounds

S.No.	Target Name	Ligand Name	Est. Free Energy Of Binding (kcal/mol)	Est. Inhibition Constant Ki (mM)	vdW+ Hbond + desolv Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Intermol. Energy (kcal/mol)	Frequency (%)	Interaction Surface
1	**VEGF**	Metformin	-4.44	0.56	-2.75	-1.69	-4.44	90	299.381
		Capsaicin	-2.8	8.9	-4.77	-0.02	-4.8	10	518.52
		Curcumin	-3.51	2.68	-5.29	0.03	-5.25	10	619.232
		Lupeol	-5.52	0.09	-6.13	0	-6.13	10	596.655
		Luteolin	-4.42	0.57	-4.43	-0.33	-4.77	60	506.586
		Sesamol	-2.55	13.46	-2.66	-0.19	-2.85	20	292.822
2	**Beta Lactamase**	Metformin	-4.37	0.63	-2.88	-1.49	-4.37	60	336.62
		Capsaicin	-3.14	5	-4.83	-0.16	-4.99	20	540.365
		Curcumin	-4.1	0.991	-5.66	-0.13	-5.79	20	541.845
		Lupeol	-5.51	0.091	-6.14	-0.02	-6.16	60	569.53
		Luteolin	-4.88	0.263	-4.85	-0.36	-5.21	30	477.153
		Sesamol	-2.62	12.01	-2.59	-0.33	-2.92	40	313.697
3	**Beta Secretase**	Metformin	-5.54	0.086	-2.56	-2.98	-5.54	80	346.622
		Capsaicin	-4.36	0.63	-6.14	0	-6.14	10	616.531
		Curcumin	-6.63	0.014	-8.37	-0.08	-8.44	30	819.406
		Lupeol	-7.64	0.003	-8.12	-0.12	-8.24	100	800.025
		Luteolin	-4.75	0.33	-4.43	-0.58	-5.01	20	604.657
		Sesamol	-3.9	1.37	-4.13	-0.07	-4.2	70	391.294
4	**CDK2**	Metformin	-3.28	3.97	-1.42	-1.86	-3.28	100	269.215
		Capsaicin	-1.7	56.87	-3.75	-0.15	-3.9	10	517.064
		Curcumin	-0.78	270.06	-1.66	-0.3	-1.97	10	317.898
		Lupeol	-3.21	4.47	-3.74	-0.09	-3.83	40	430.992
		Luteolin	-2.2	24.25	-2.01	-0.48	-2.48	10	297.623
		Sesamol	-2.11	28.53	-2.05	-0.36	-2.41	40	238.029
5	**Fructose-1,6-bisphosphatase**	Metformin	-2.93	7.15	-1.54	-1.39	-2.93	20	219.69
		Capsaicin	-4.08	1.03	-6.25	-0.15	-6.4	10	653.55
		Curcumin	-4.3	0.71	-5.84	-0.17	-6.01	10	672.827
		Lupeol	-6.37	0.022	-6.97	-0.04	-7.02	10	689.31
		Luteolin	-4.94	0.24	-5.12	-0.06	-5.18	20	556.009
		Sesamol	-3.2	4.55	-3.36	-0.14	-3.49	10	375.807
6	**HSP90**	Metformin	-4.09	1.01	-3.58	-0.5	-4.09	30	365.345
		Capsaicin	-2.24	22.75	-5.45	0.03	-5.42	10	731.505

		Curcumin	-0.72	298.65	-3.5	-0.03	-3.53	10	743.659
		Lupeol	2.75		2.12	-0.2	1.93	70	821.826
		Luteolin	-3.88	1.43	-4.22	-0.17	-4.4	20	662.859
		Sesamol	-3.83	1.55	-4.04	-0.09	-4.13	60	373.835
7	**Insulin Receptor**	Metformin	-3.25	4.18	-2.11	-1.14	-3.25	20	337.133
		Capsaicin	-2.33	19.76	-4.66	0.11	-4.55	10	543.077
		Curcumin	-3.09	5.47	-4.87	0.08	-4.79	10	530.004
		Lupeol	-5.57	0.083	-6.18	0	-6.19	60	554.214
		Luteolin	-3.31	3.72	-3.23	-0.37	-3.6	40	447.565
		Sesamol	-2.94	7	-3.09	-0.14	-3.24	90	325.973
8	**Neprilysin**	Metformin	-4.06	1.05	-2.2	-1.86	-4.06	60	309.984
		Capsaicin	-2.62	12.04	-4.82	0.05	-4.78	10	559.056
		Curcumin	-3.25	4.12	-4.93	-0.04	-4.97	30	589.278
		Lupeol	-5.41	0.109	-5.97	-0.03	-6	60	562.791
		Luteolin	-4.45	0.545	-4.39	-0.27	-4.66	20	457.064
		Sesamol	-2.5	14.58	-2.61	-0.19	-2.8	80	300.272
9	**Thymidylate Synthase**	Metformin	-4.22	0.8	-2.86	-1.36	-4.22	100	394.422
		Capsaicin	-4.13	0.93	-6.15	0.09	-6.06	10	680.201
		Curcumin	14.03		11.83	-0.06	11.77	30	748.321
		Lupeol	-2.65	11.43	-3.16	-0.09	-3.25	30	801.617
		Luteolin	-5.14	0.17	-5.04	-0.4	-5.44	70	628.418
		Sesamol	-3.5	2.73	-3.39	-0.41	-3.8	50	371.343
10	**Phosphotyrosine protein phosphatase**	Metformin	-1.98	35.18	-1.19	-0.79	-1.98	10	311.765
		Capsaicin	378.53	-	357.27	0.17	357.44	20	547.094
		Curcumin	691.15	-	686.56	0.01	686.57	10	691.823
		Lupeol	1.49E+03	-	1.49E+03	-0.01	1.49E+03	100	671.526
		Luteolin	193.1	-	192.42	0.14	192.56	30	527.799
		Sesamol	-2.92	7.21	-3.09	-0.13	-3.22	70	338.348
11	**Phosphoinositol dependent kinase (PDK)**	Metformin	-3.65	2.11	-1.85	-1.8	-3.65	40	413.603
		Capsaicin	-3.24	4.22	-5.58	-0.08	-5.65	10	646.048
		Curcumin	-3.13	5.08	-4.58	-0.33	-4.91	10	735.638
		Lupeol	-5.39	0.113	-6.12	-0.02	-6.14	50	665.328
		Luteolin	-4.35	0.652	-4.57	-0.08	-4.65	30	562.545
		Sesamol	-3.2	4.54	-3.3	-0.2	-3.49	40	377.914
12	**Thymidine Kinase**	Metformin	-3.65	2.11	-2.89	-0.76	-3.65	100	412.306
		Capsaicin	-6.38	0.021	-9	-0.32	-9.32	20	782.392
		Curcumin	-6.23	0.027	-8.69	-0.01	-8.7	20	837.269
		Lupeol	-6.48	0.018	-7.01	-0.07	-7.08	100	822.019
		Luteolin	-6.68	0.013	-6.6	-0.37	-6.97	90	659.429
		Sesamol	-4.55	0.462	-4.75	-0.1	-4.85	20	385.53
13	**Tyrosine**	Metformin	-4.82	0.296	-2.48	-2.33	-4.82	20	348.054

	Kinase	Capsaicin	-4.89	0.261	-7.21	-0.17	-7.38	10	783.76
		Curcumin	-5.28	0.136	-6.85	-0.1	-6.95	20	809.323
		Lupeol	-9.55	99.62nm	-10.17	-0.11	-10.27	80	878.554
		Luteolin	-6.58	0.015	-6.63	-0.29	-6.92	60	666.861
		Sesamol	-3.6	2.31	-3.85	-0.04	-3.9	50	393.601
14	**cAMP dependent protein kinase (PKA)**	Metformin	-4.36	0.641	-2.62	-1.74	-4.36	100	281.489
		Capsaicin	-2.47	15.37	-4.78	0	-4.78	10	505.776
		Curcumin	-3.36	3.44	-4.43	-0.11	-4.53	10	510.662
		Lupeol	-5.04	0.204	-5.64	0	-5.64	10	748.338
		Luteolin	-3.86	1.47	-3.76	-0.3	-4.06	60	409.433
		Sesamol	-3.53	2.58	-3.81	-0.02	-3.83	30	369.851
15	**PPAR**	Metformin	-3.91	1.37	-1.73	-2.17	-3.91	10	320.497
		Capsaicin	-5.86	0.051	-8.14	-0.08	-8.22	10	999.94
		Curcumin	-6.81	0.01	-8.78	0.06	-8.72	10	1120.172
		Lupeol	-9.09	218.14 nm	-9.68	-0.01	-9.69	100	1117.017
		Luteolin	-6.89	0.008	-6.84	-0.41	-7.25	80	898.872
		Sesamol	-3.79	1.65	-4.06	-0.03	-4.09	50	387.659
16	**eNOS**	Metformin	-6.82	0.01	-3.68	-3.13	-6.82	100	419.151
		Capsaicin	-5.78	58.19um	-8.01	-0.43	-8.44	30	814.213
		Curcumin	-5.45	0.1	-6.79	-0.05	-6.84	20	849.887
		Lupeol	-8.15	1.06um	-8.69	-0.11	-8.8	70	863.581
		Luteolin	-6.53	16.32 um	-6.23	-0.68	-6.9	60	658.101
		Sesamol	-5.6	78.82 um	-5.58	-0.31	-5.9	100	387.685
17	**Acetylcholinesterase**	Metformin	-0.05	926.71	0	-0.05	-0.05	30	321.77
		Capsaicin	0.91	-	0	0	0	10	503.016
		Curcumin	1.11	-	0	0	0	10	690.424
		Lupeol	0.59	-	0	0	0	10	672.07
		Luteolin	0.14	-	0	0	0	10	486.079
		Sesamol	0.3	-	0	0	0	20	305.205
18	**Glutathione S Transferase**	Metformin	-0.03	944.98	0	-0.03	-0.03	40	10475.75
		Capsaicin	0.89	-	0	0	0	10	10475.75
		Curcumin	1.1	-	0	0	0	10	10475.75
		Lupeol	0.59	-	0	0	0	10	10475.75
		Luteolin	0.14	-	0	0	0	10	10475.75
		Sesamol	0.3	-	0	0	0	10	10475.75
19	**GSK 3β**	Metformin	0.06		0	0.06	0.06	90	15959.16
		Capsaicin	0.89		0	0	0	10	502.766
		Curcumin	1.11		0	0	0	10	15959.16
		Lupeol	0.59		0	0	0	10	15959.16
		Luteolin	0.14		0	0	0	10	15959.16
		Sesamol	0.3		0	0	0	10	15959.16

20	**MAP Kinase**	Metformin	0	-	0	0	0	70	321.781
		Capsaicin	0.89	-	0	0	0	10	506.744
		Curcumin	1.11	-	0	0	0	10	690.488
		Lupeol	0.59	-	0	0	0	20	672.051
		Luteolin	0.14	-	0	0	0	10	486.136
		Sesamol	0.3	-	0	0	0	40	305.186

The docking results and HB plot results for interacting residues are summarized in **Figures 14-29**.

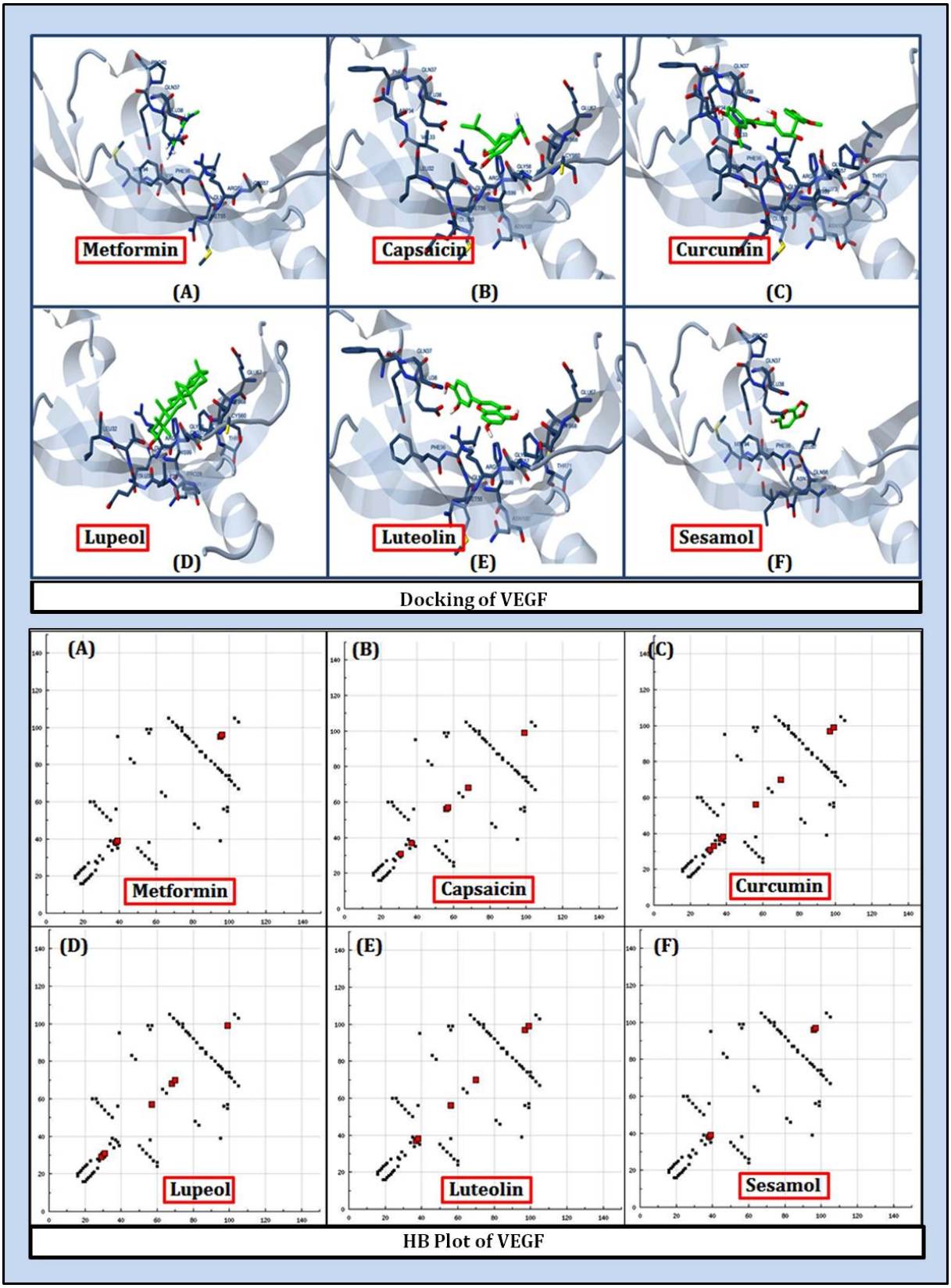

Figure 14: Docking and HB plot of VEGF

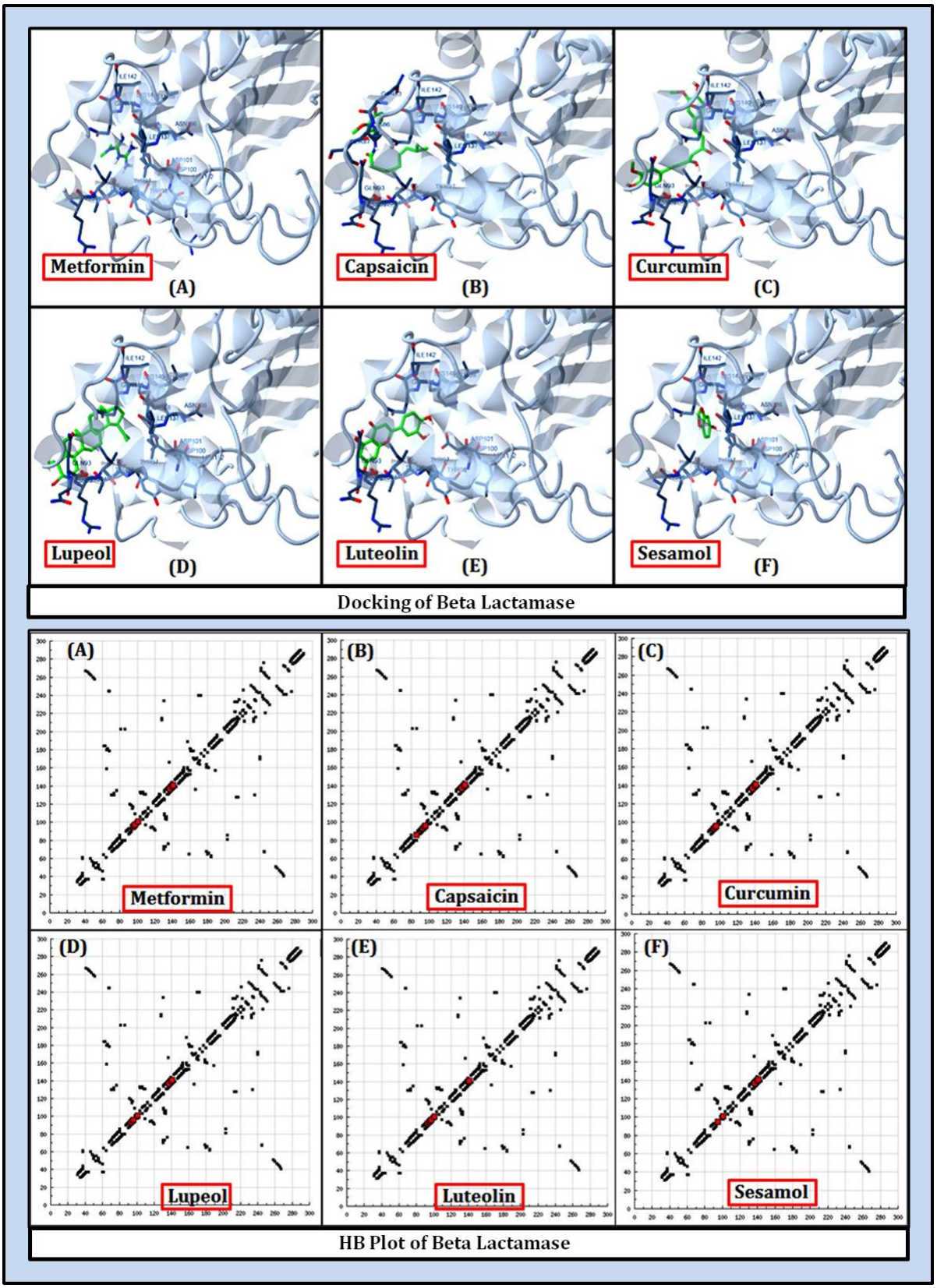

Figure 15: Docking and HB plot of Beta Lactamase

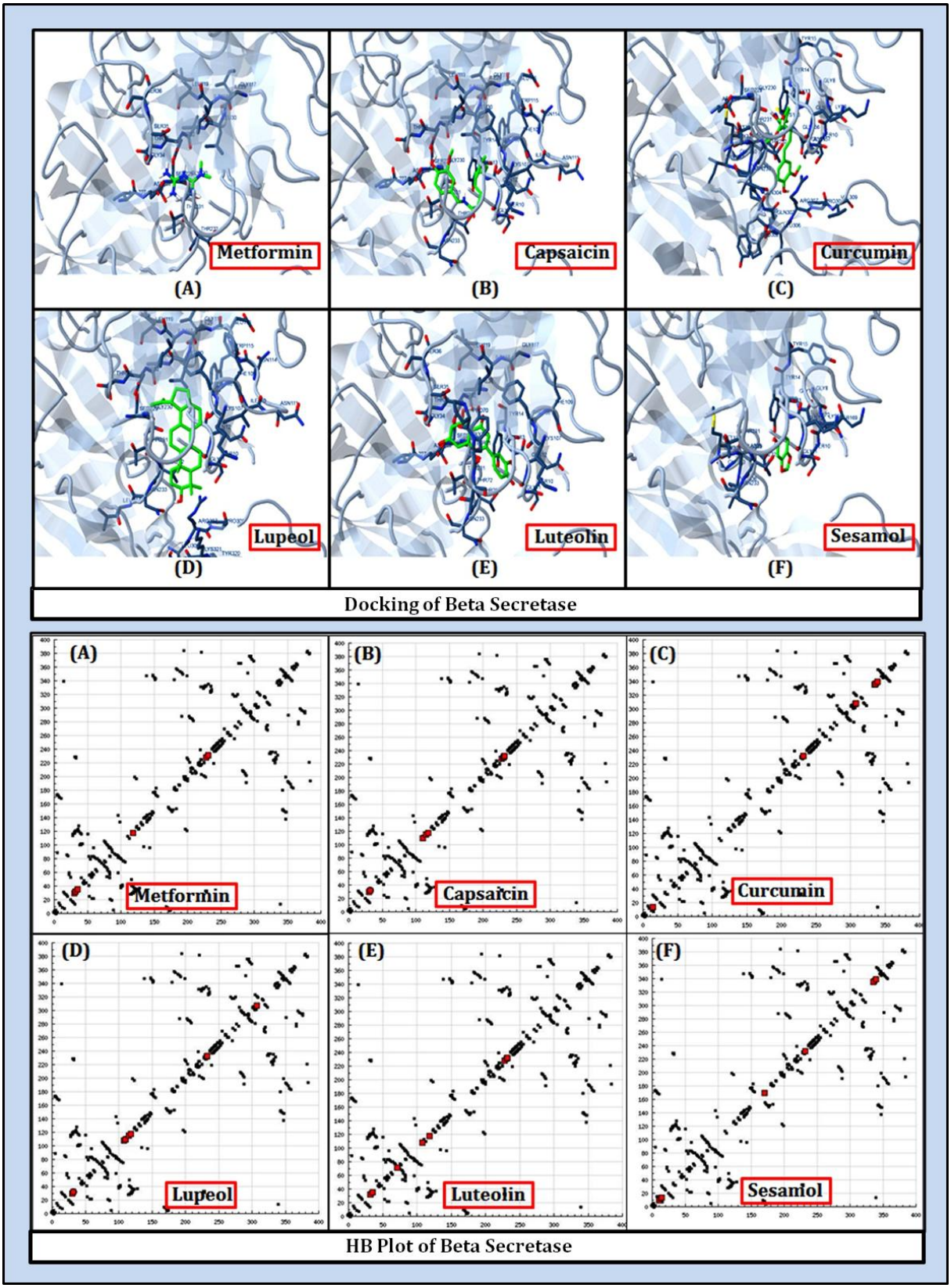

Figure 16: Docking and HB plot of Beta Secretase

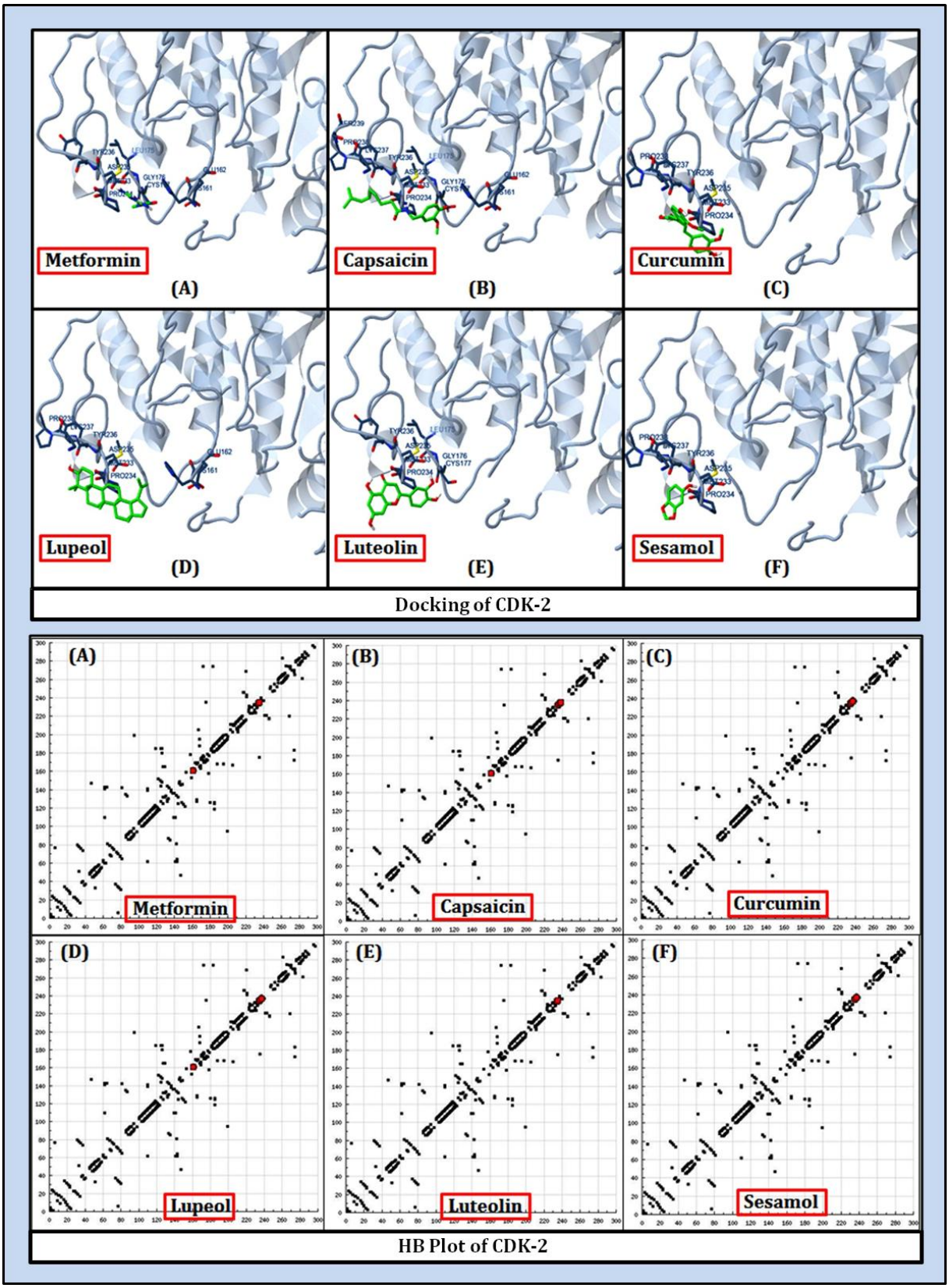

Figure 17: Docking and HB plot of CDK 2

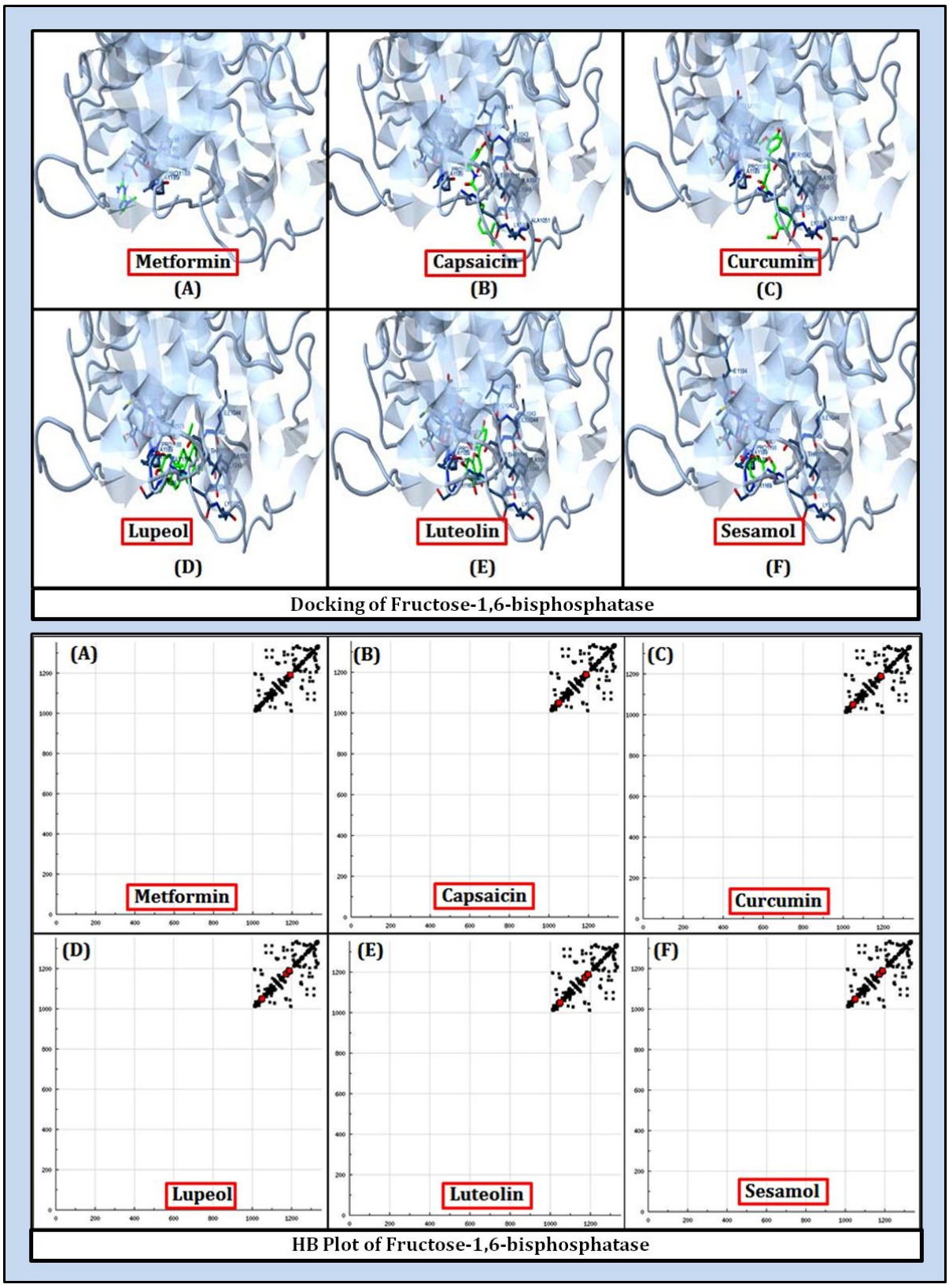

Figure 18: Docking and HB plot of Fructose-1,6-bisphosphatase

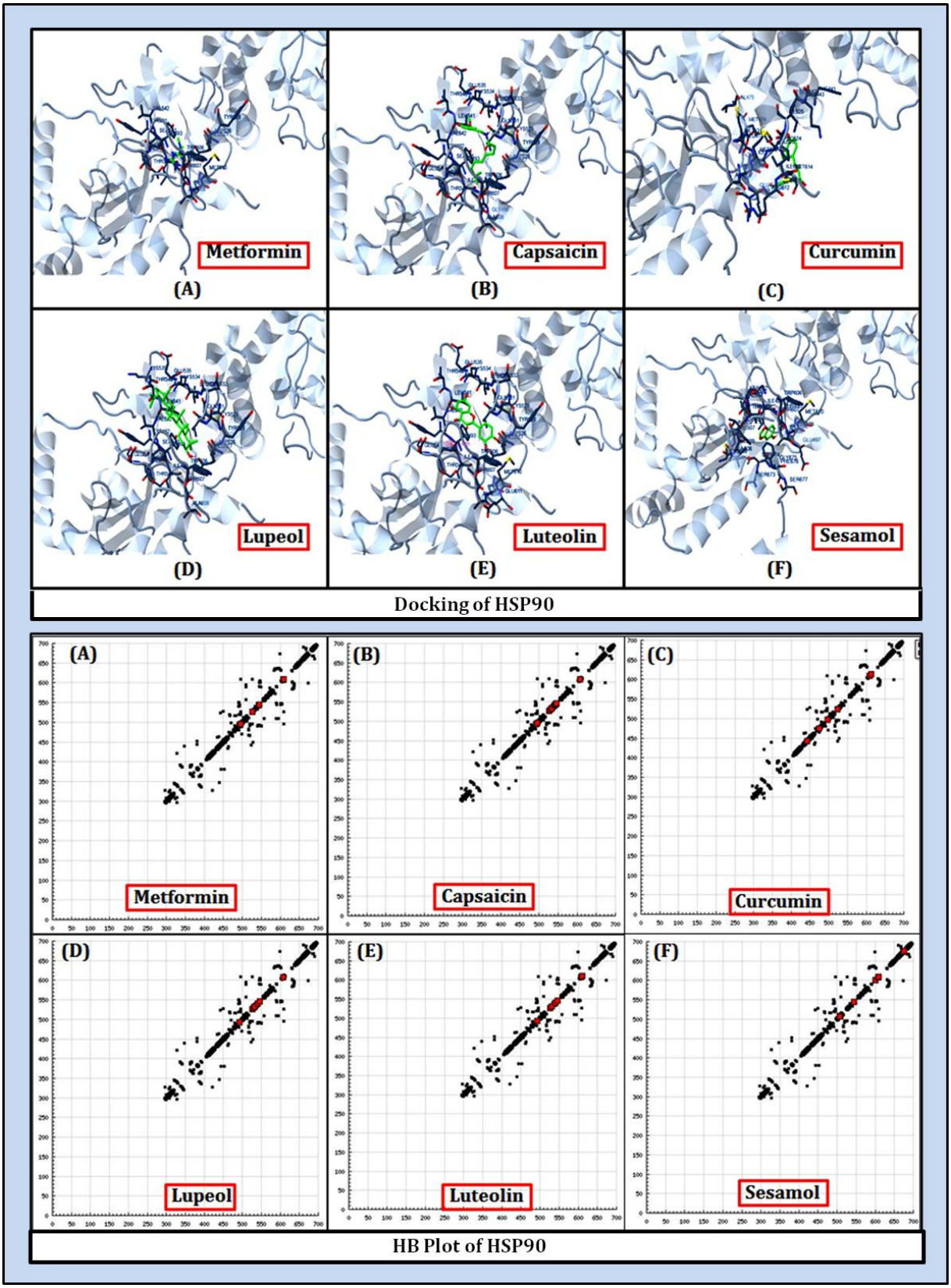

Figure 19: Docking and HB plot of HSP 90

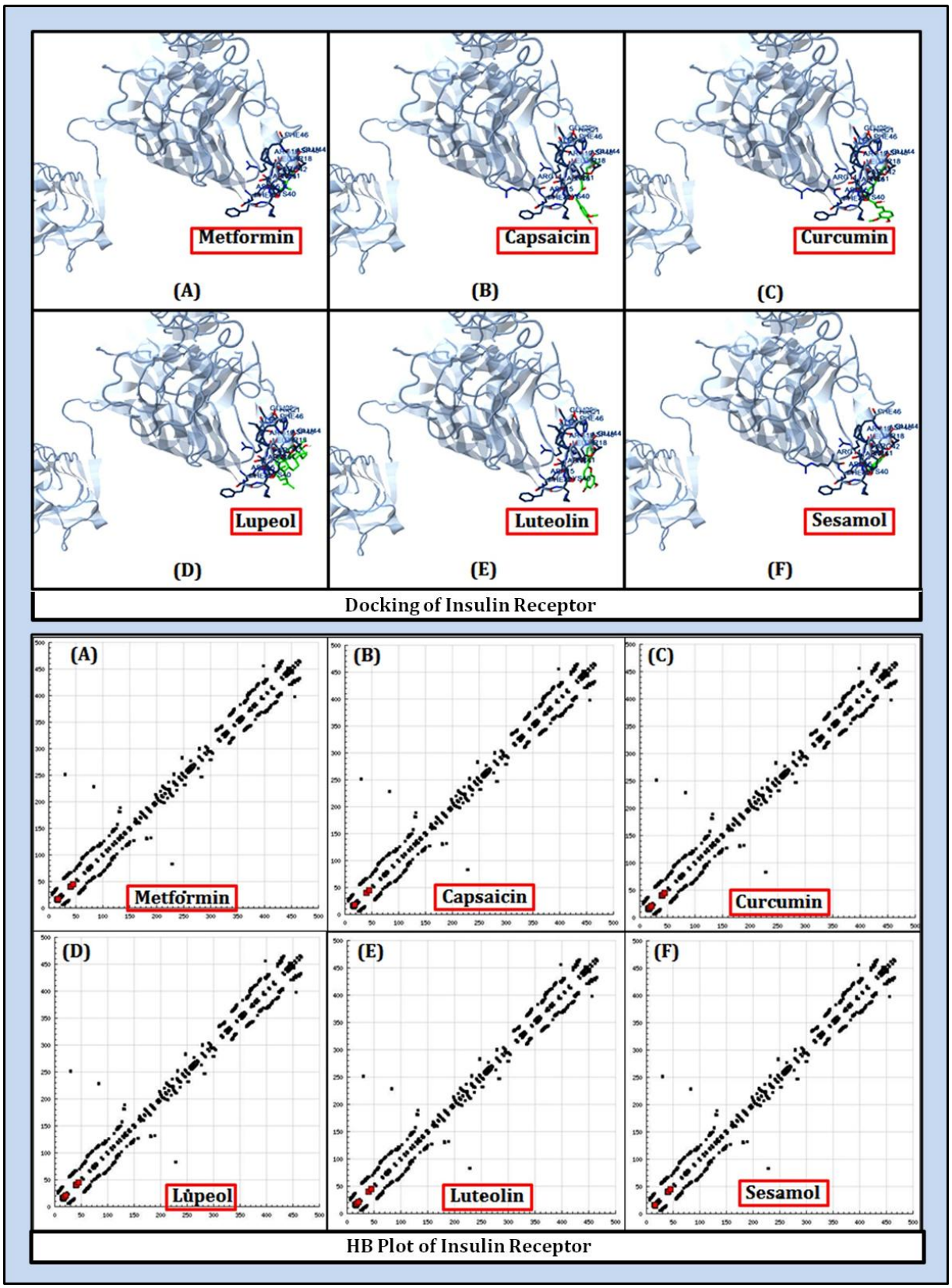

Figure 20: Docking and HB plot of Insulin Receptor

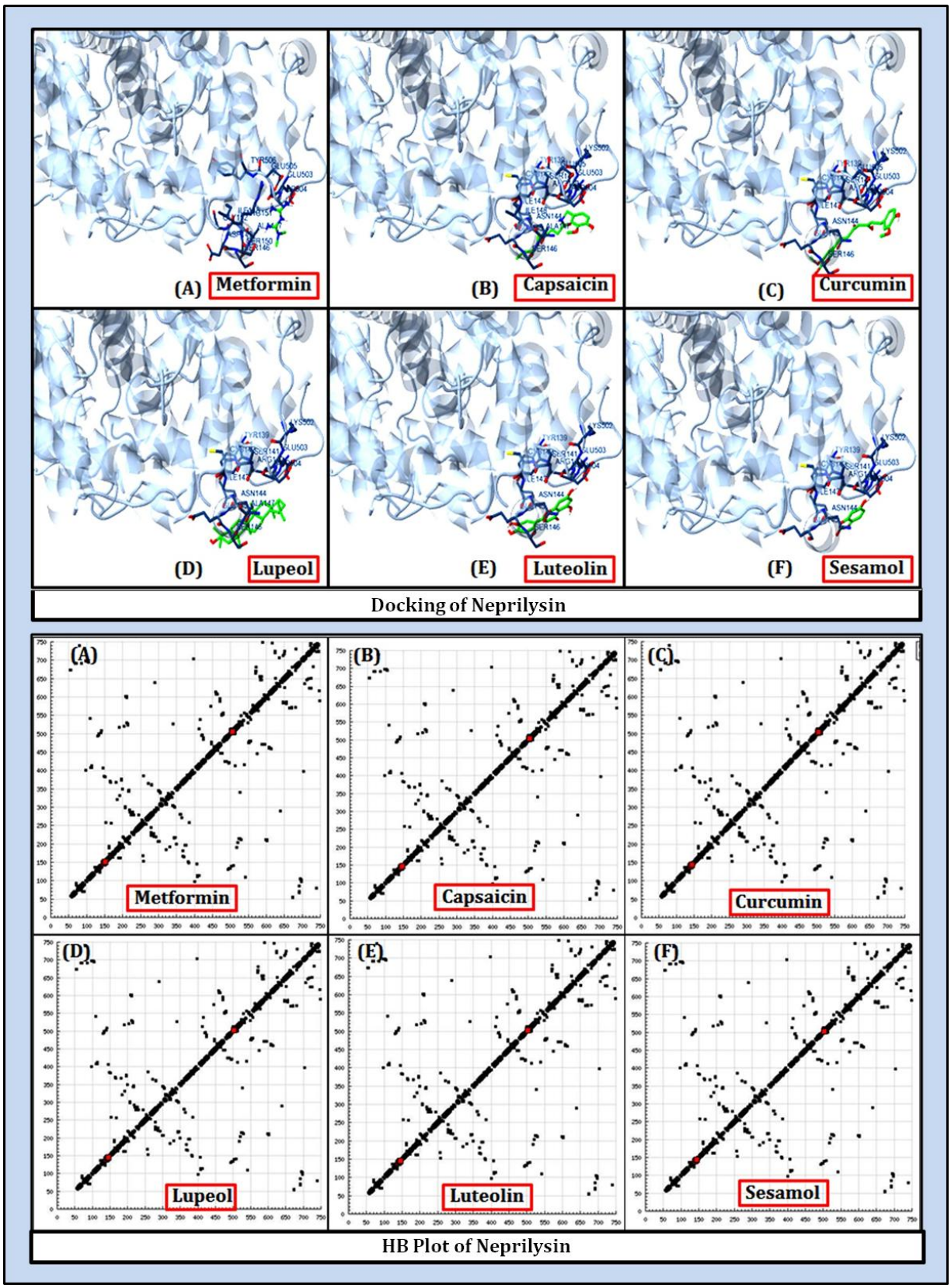

Figure 21: Docking and HB plot of Neprilysin

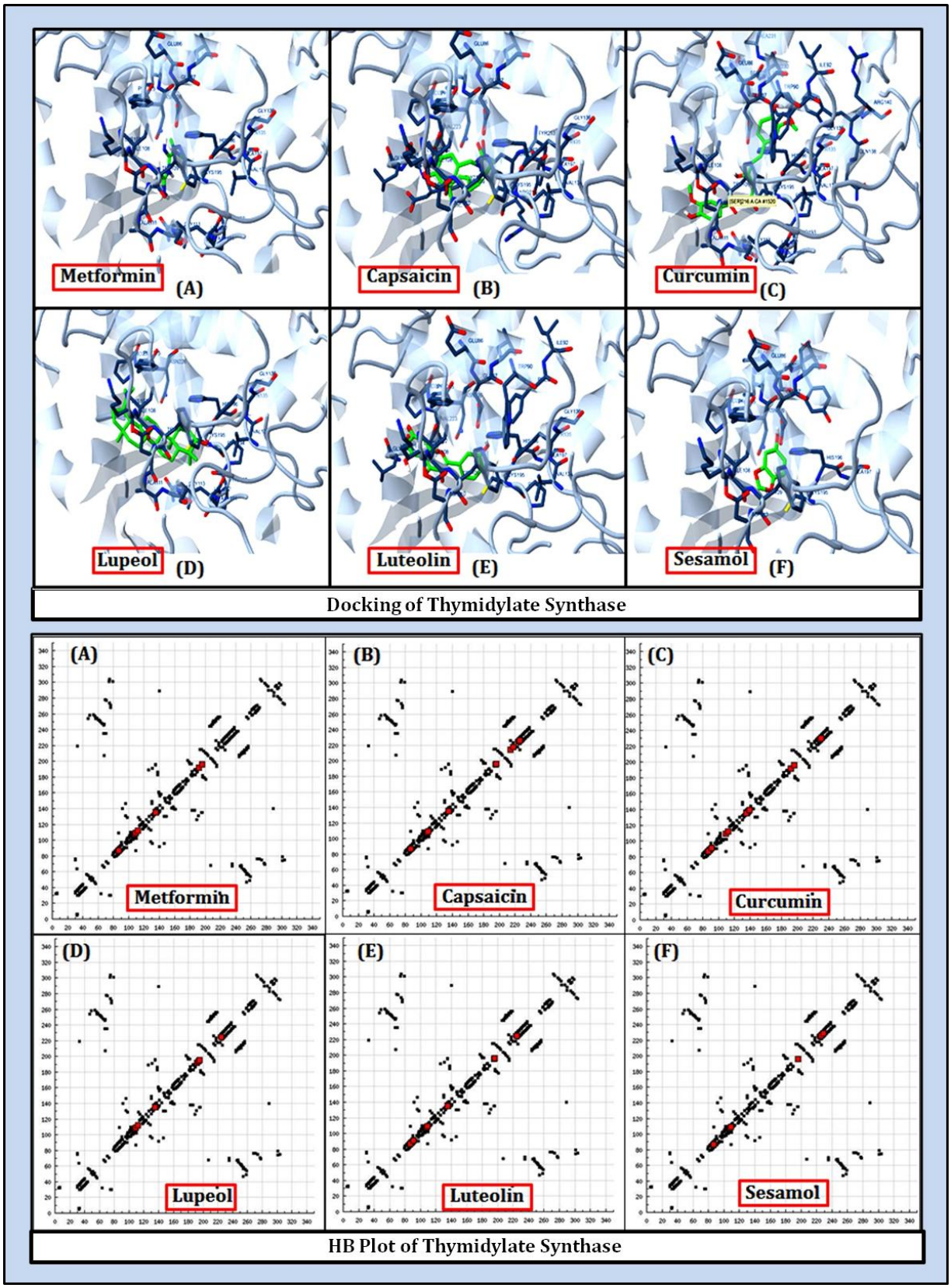

Figure 22: Docking and HB plot of Thymidylate Synthase

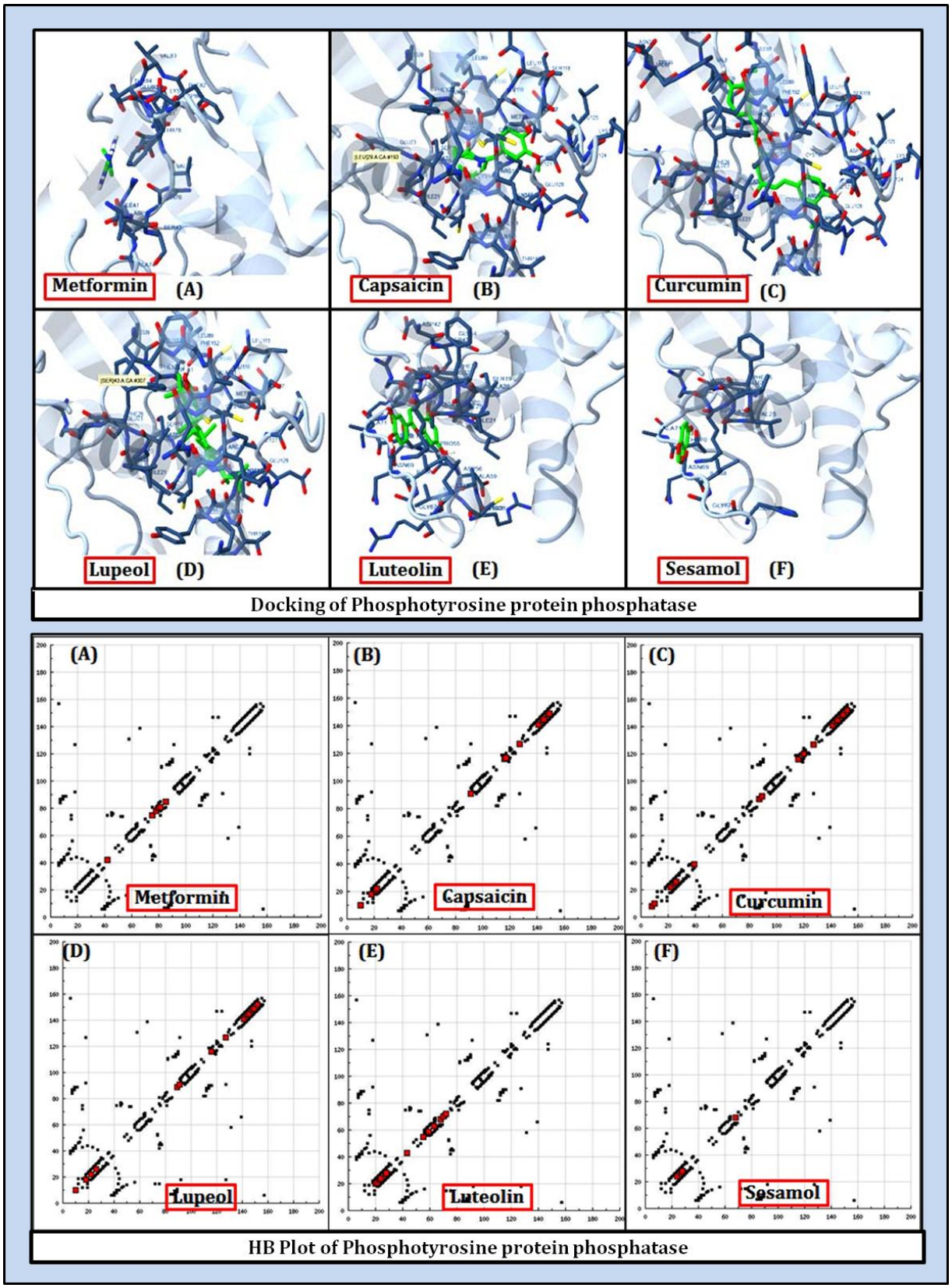

Figure 23: Docking and HB plot of Phosphotyrosine protein phosphatase

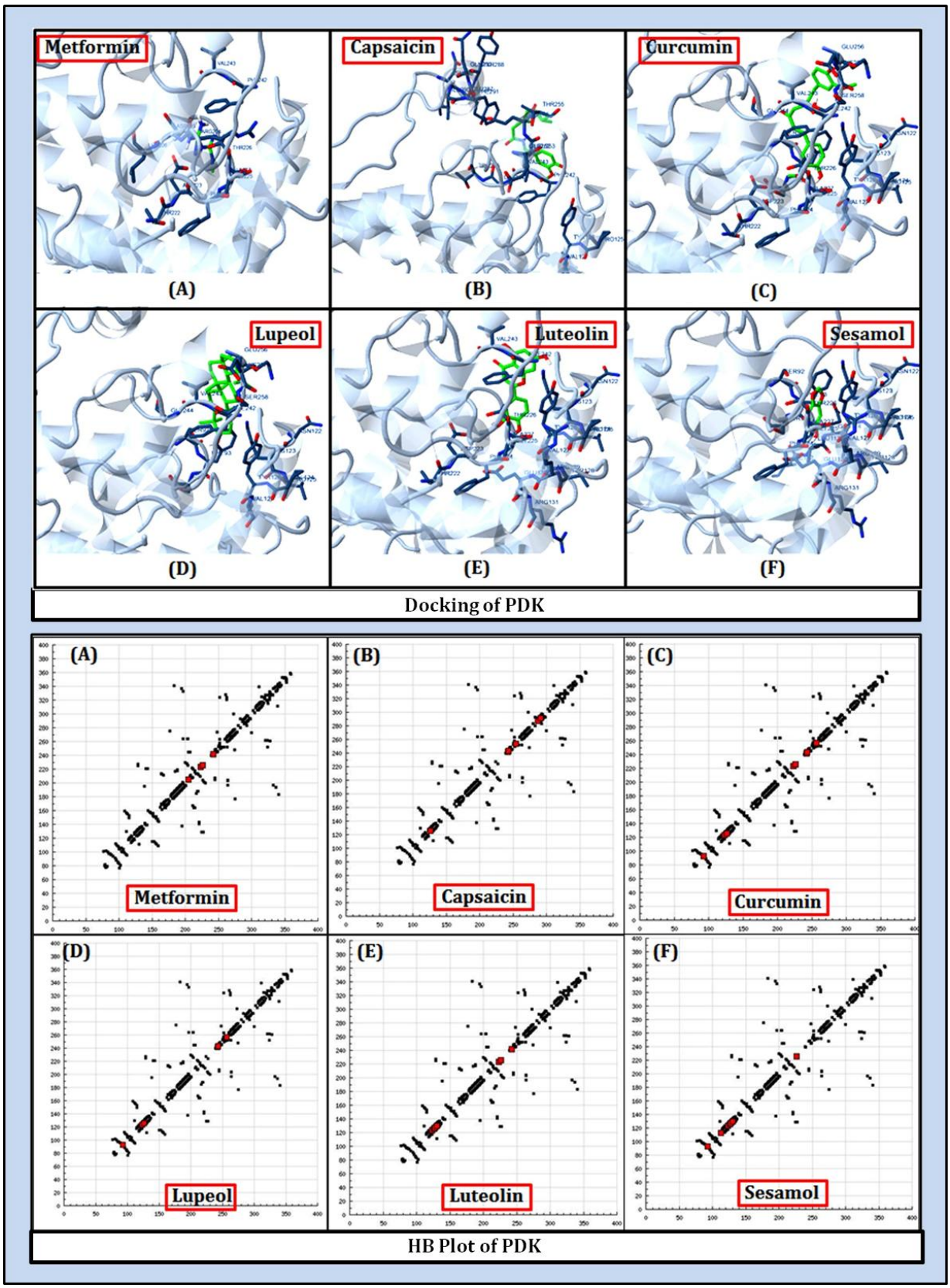

Figure 24: Docking and HB plot of PDK

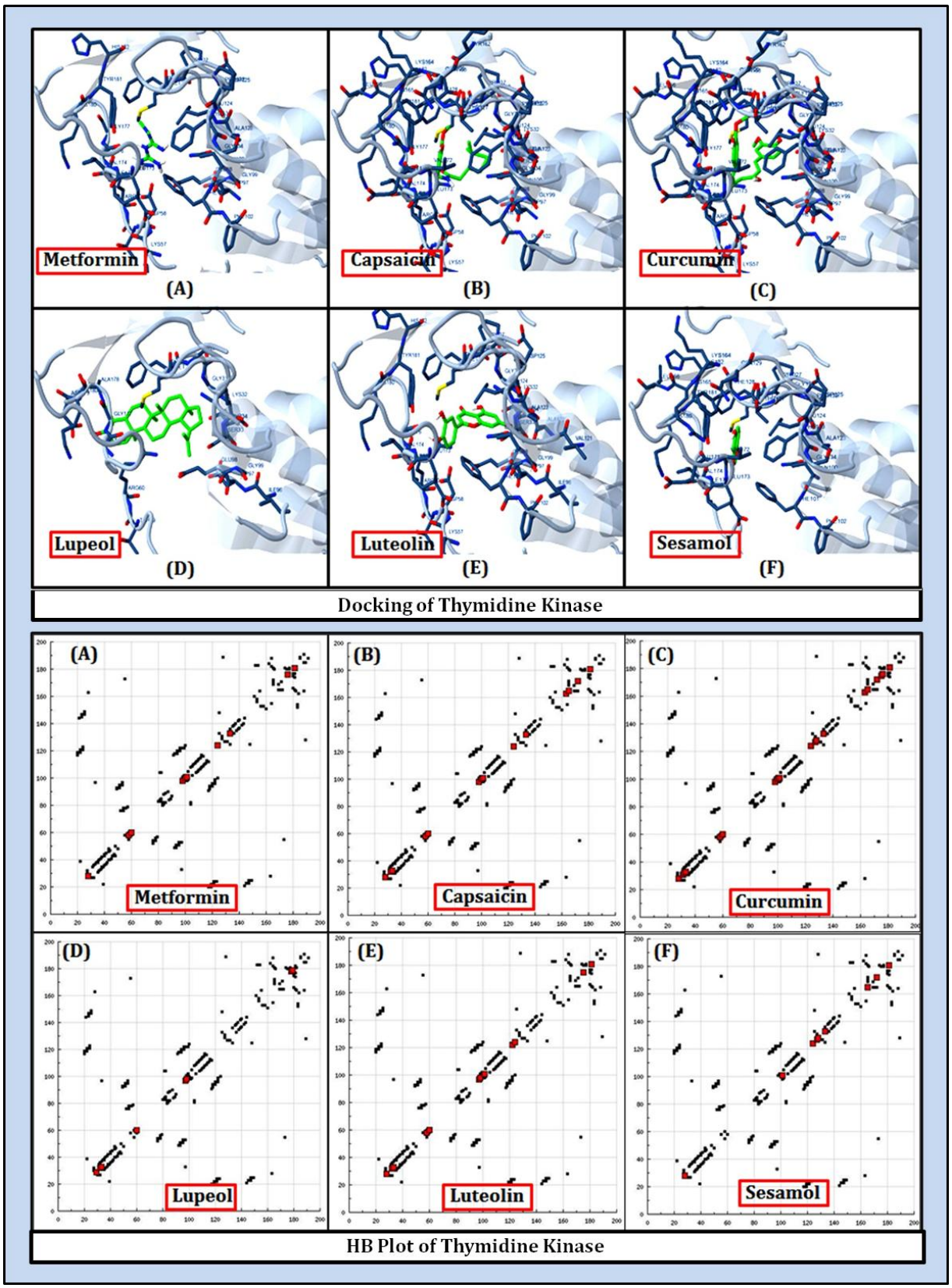

Figure 25: Docking and HB plot of Thymidine Kinase

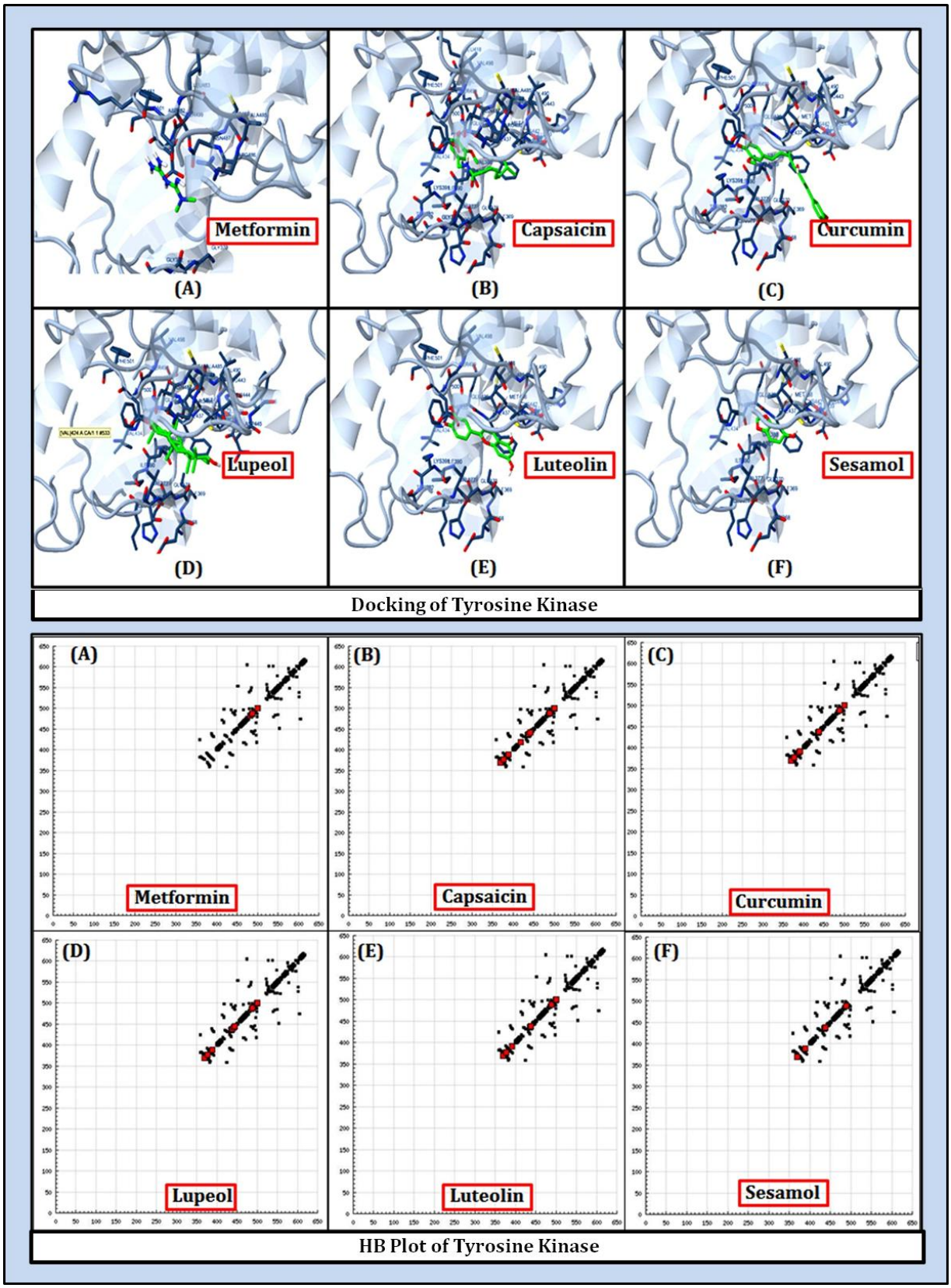

Figure 26: Docking and HB plot of Tyrosine Kinase

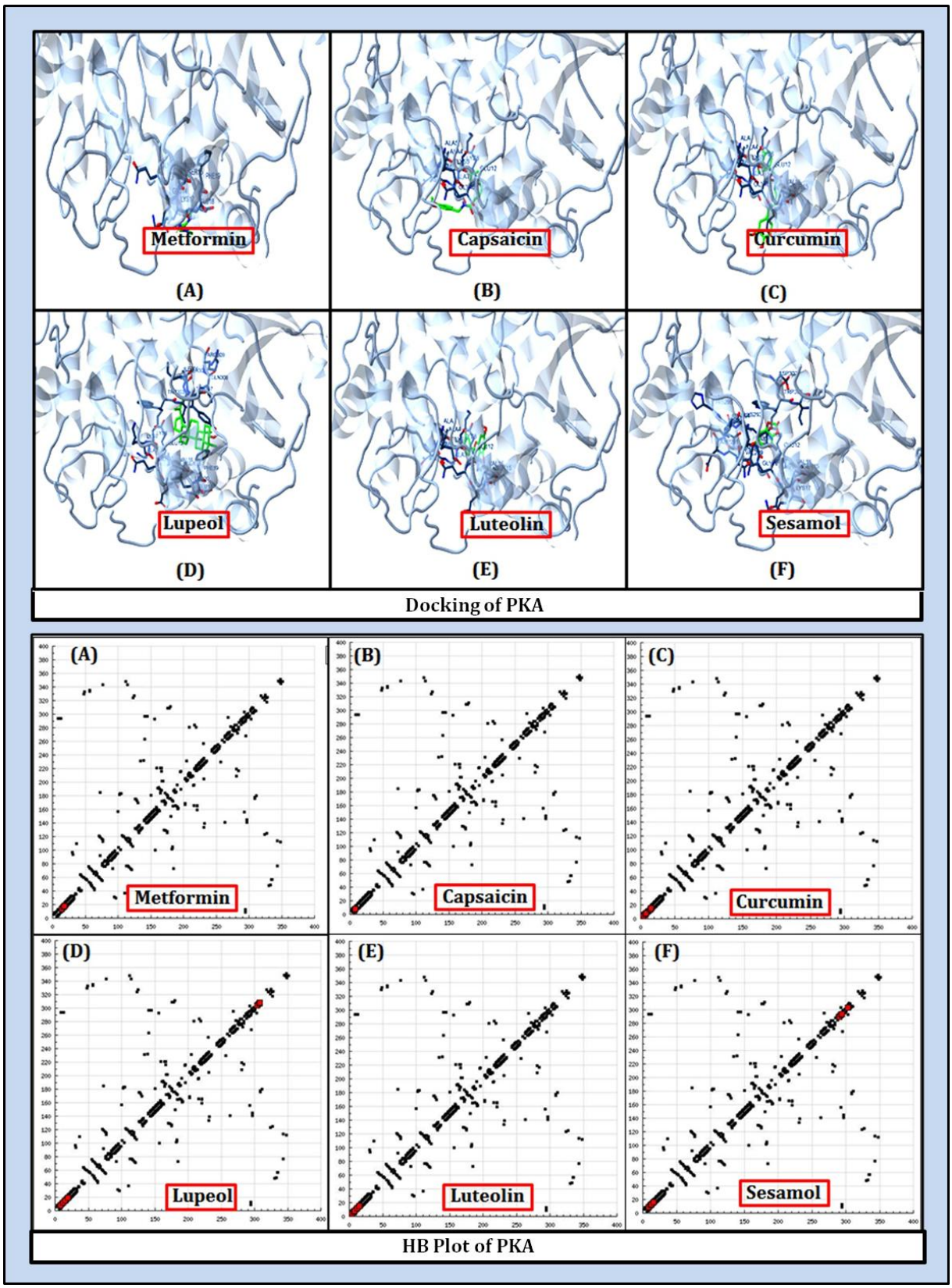

Figure 27: Docking and HB plot of PKA

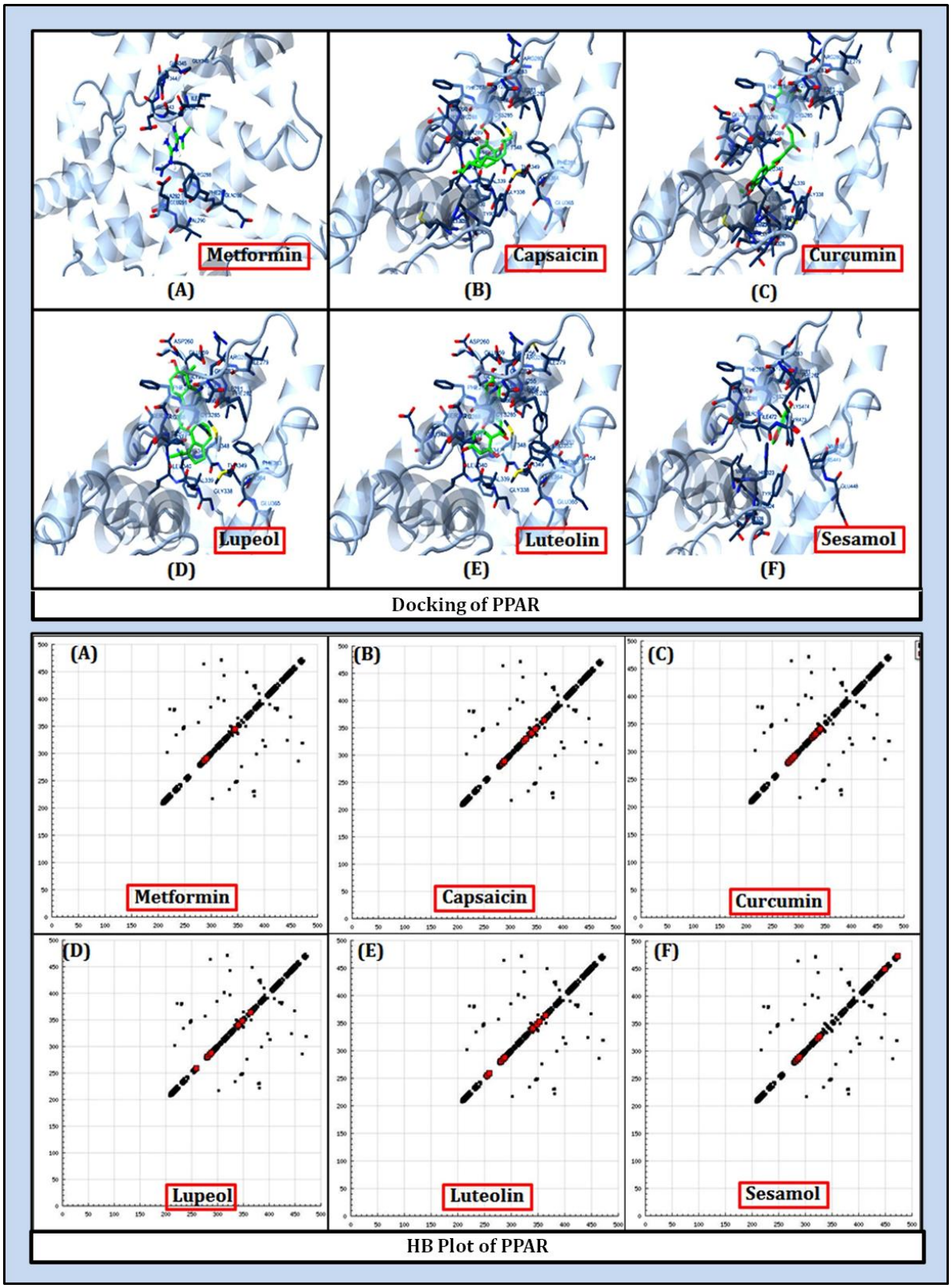

Figure 28: Docking and HB plot of PPAR

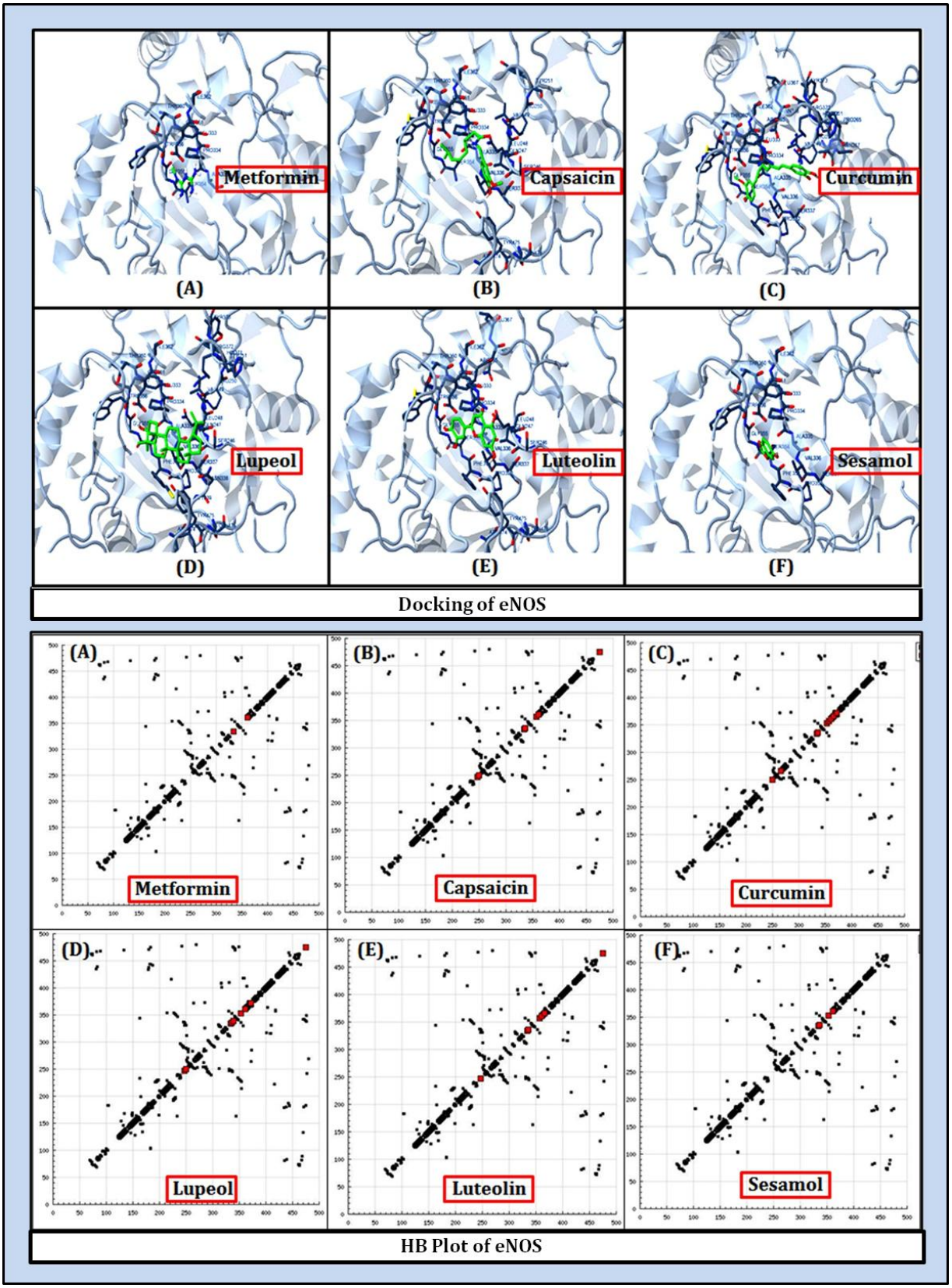

Figure 29: Docking and HB plot of eNOS

The results from the docking analysis have been comparatively analyzed and summarized in **Table 8**. The most effective drug with the minimum free energy of binding was identified to be Lupeol out of the five ayurvedic drugs.

Table 8: The summary docking values of all the targets with most effective ayurvedic drug based on minimum values of the energies

S. No.	Target Name	Effective Ayurvedic Drug	Est. Free Energy Of Binding Of Control (kcal/mol)	Est. Free Energy Of Binding Of Ayurvedic Drug (kcal/mol)
1	VEGF	Lupeol	-4.44	-5.52
2	Beta Lactamase	Lupeol	-4.37	-5.51
3	Beta Secretase	Lupeol	-5.54	-7.64
4	CDK2	Lupeol	-3.28	-3.21
5	Fructose-1,6-bisphosphatase	Lupeol	-2.93	-6.37
6	HSP90	Luteolin	-4.09	-3.88
7	Insulin Receptor	Lupeol	-3.25	-5.57
8	Neprilysin	Lupeol	-4.06	-5.41
9	Thymidylate Synthase	Luteolin	-4.22	-5.14
10	Phosphotyrosine protein phosphatase	Sesamol	-1.98	-2.92
11	Phosphoinositol dependent kinase (PDK)	Lupeol	-3.65	-5.39
12	Thymidine Kinase	Luteolin	-3.65	-6.68
13	Tyrosine Kinase	Lupeol	-4.82	-9.55
14	cAMP dependent protein kinase (PKA)	Lupeol	-4.36	-5.04
15	PPAR	Lupeol	-3.91	-9.09
16	eNOS	Lupeol	-6.82	-8.15

5.5 COMPARATIVE ANALYSIS

The residues of Metformin and the most effective ayurvedic drug have been summarized in **Table 9**. The combined docking results and interacting residues of the most effective ayurvedic drug are summarized in **Figure 30**.

Table 9: The summary of common interacting residues of metformin (control) and the most effective ayurvedic drug

SNo.	Target Protein	Int. Residues Of Control Drug (Metformin)	Potential Test Drug	Int. Residues Of Potential Test Drug	Common int. residues
1	VEGF	GLU38,TYR39,SER95,PHE96	Lupeol	ILE29,THR31,CYS57,CYS68,PRO70,HIS99	-
2	β-Lactamase	ILE95,THR96,TYR97,ASP101,LEU137,LYS140,GLN141	Lupeol	GLN93,ILE95,THR96,ASP101,LEU137,LYS140,GLN141	ILE95,THR96,ASP101,LEU137,LYS140,GLN141
3	β-Secretase	LEU30,ASP32,SER35,ILE118,ASP228,THR231	Lupeol	LEU30,ASP32,PHE108,ILE110,TRP115,ILE118,THR232,ASN233,ARG307	LEU30,ASP32,ILE118
4	CDK2	HIS161,PRO234,ASP235	Lupeol	HIS161,PRO234,ASP235,LYS237	HIS161,PRO234,ASP235
5	Fructose-1,6-bisphosphatase	ASP1187,PRO1188,ALA1189,ILE1190	Lupeol	SER1045,THR1046,ARG1049,THR1171,LEU1186,PRO1188	PRO1188
6	HSP90	TYR493,ILE494,THR495,GLU527,SER543,VAL544,THR545.THR607,ASN609	Luteolin	TYR493,GLU527,VAL530,LYS534,THR540,LEU541,LYS546,THR607	TYR493,GLU527,THR607
7	Insulin Receptor	ASN16,LEU17,LYS40,THR41,ASP45	Lupeol	ASN16,LEU17,THR18,HIS21,LYS40,THR41,ASP45	ASN16,LEU17,LYS40,THR41,ASP45
8	Neprilysin	ALA147,SER150,ARG151,ASP504,GLU505	Lupeol	ARG140,ILE143,ASN144,GLU145,SER146,GLU503	-
9	Thymidylate Synthase	GLU87,ILE108,TRP109,ASN112,TYR135,LEU192,HIS196	Luteolin	GLU87,PHE91,ILE108,TRP109,TYR135,CYS195,HIS196,PHE225	GLU87,ILE108,TRP109,TYR135,HIS196
10	Phosphotyrosine protein phosphatase	ASP42,ARG75,THR78,GLU80,ASP81,PHE85	Sesamol	ALA24,ARG27,LYS28,ILE68	-
11	Phosphoinositol dependent kinase (PDK)	ASP205,ASP223,THR226,PHE242	Lupeol	PHE93,LYS123,TYR126,PHE242,VAL243,LYS257	PHE242

12	Thymidine Kinase	MET28,ASP58,ARG60,GLU98,GLN100,PHE101,LEU124,PHE133,GLY176,TYR181	Luteolin	MET28,LYS32,SER33,ASP58,ARG60,ASP97,GLU98,PHE101,ALA122,LEU124,ILE175,TYR181	MET28,ASP58,ARG60,GLU98,PHE101,LEU124,TYR181
13	Tyrosine Kinase	ASP482,ARG486,ASN487,ASP500	Lupeol	ILE369,VAL377,ALA389,PHE435,PHE437,MET438,CYS442,ASP445,ARG486,LEU489,SER499,ASP500	ARG486,ASP500
14	cAMP dependent protein kinase	GLU14,LYS17,GLU18	Lupeol	LYS8,GLU12,SER15,PHE19,ILE304,TYR307,GLN308	-
15	PPAR	PHE287,GLU291,SER342,GLU343,GLN345	Lupeol	GLU259,ARG280,ILE281,CYS285,ARG288,VAL339,ILE341,MET348,MET364	-
16	eNOS	PRO334,GLU361	Lupeol	GLN247,ARG250,PRO334,VAL336,MET339,PHE353,GLU361,ARG372,TYR475	PRO334,GLU361

Since, Lupeol has most negative energy of binding with most of the targets, it can therefore be used as a potential drug for ameliorating the effect of AD caused by the progression of T2DM.

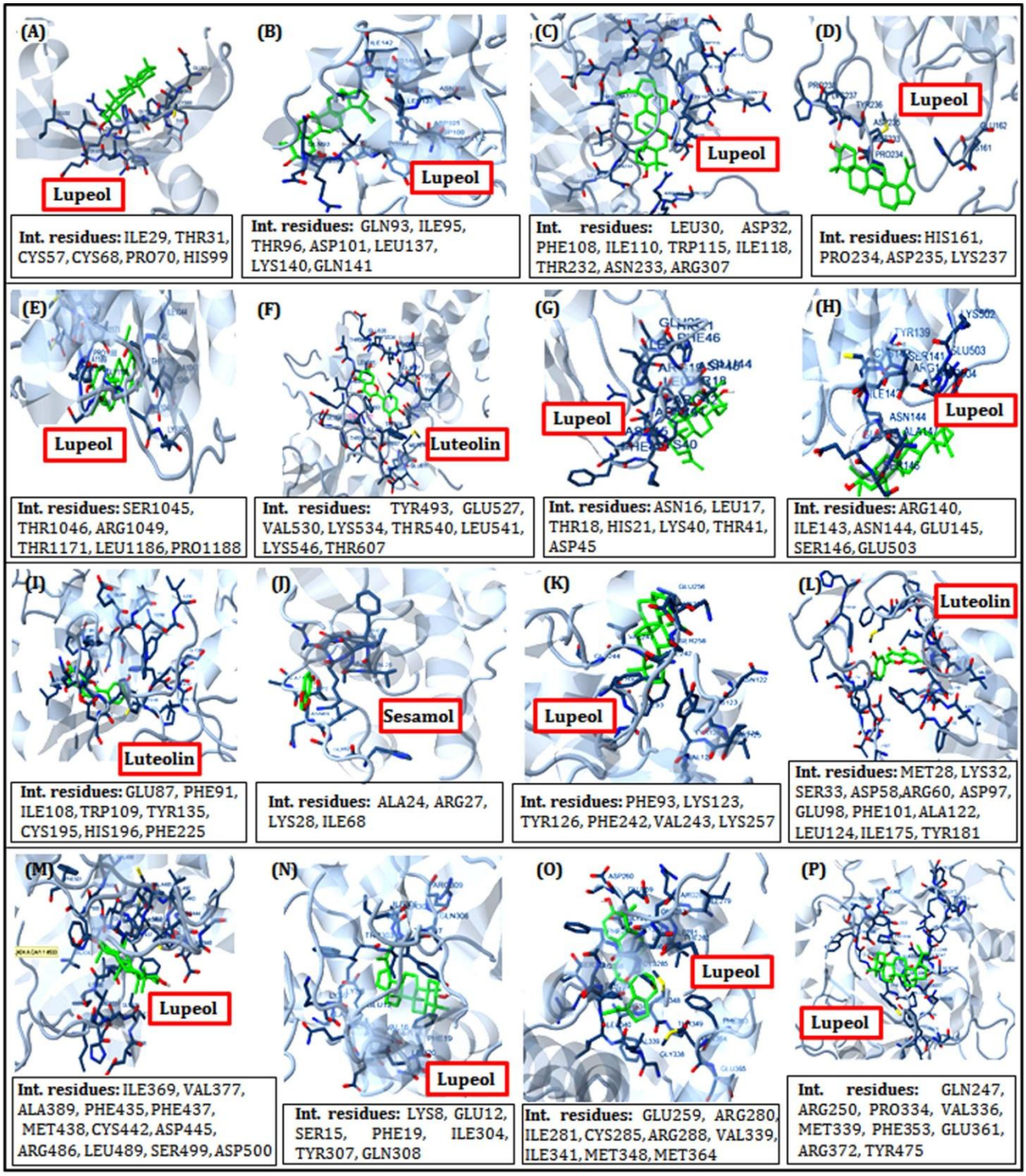

Figure 30: Interacting residues and docking results of the most effective drug with all the screened targets; (A) VEGF, (B) Beta Lactamase, (C) Beta Secretase, (D) CDK 2, (E) Fructose-1,6-bisphosphatase, (F) HSP 90, (G) Insulin Receptor, (H) Neprilysin, (I) Thymidylate Synthase, (J) Tyrosine protein phosphatase, (K) PDK, (L) Thymidine Kinase, (M) Tyrosine Kinase, (N) PKA, (O) PPAR, (P) eNOS

6. CONCLUSION

Type-3 Diabetes is a term that proposes the emerging link from T2DM to AD. Traditionally, both of these diseases were considered independently. But, now-a-days, there are a variety of cross-sectional studies showing connections that relate AD and T2DM as both are metabolic disorders associated with aging and thus increasing the possibility of risk development of each other. Some common risk factors include obesity, sedentary habits, hyperinsulinemia and insulin resistance. Several mechanisms have been proposed but it is believed that insulin resistance, inflammation and altered insulin signaling are the key culprits that attenuate the disease progression from T2DM to AD. The drugs that are available in market for AD have not shown an effective treatment due to their side effects. These side effects slowly worsen the diseased condition ultimately leading to death of the patient. Due to this reason, we chose ayurvedic drugs curcumin, capsaicin, lupeol, luteolin and sesamol for treating and preventing the disease taking Metformin as control. All these ayurvedic drugs were docked with the key targets that were known to have role in key role in linking these disorders. The binding affinity and active site residues of these drugs were analyzed and compared with the already given drug Metformin. Out of the five ayurvedic drugs chosen, it was depicted that Lupeol has the most negative binding energy values such as -9.09 kcal/mol in case of PPAR (Metformin -3.91 kcal/mol) and -9.55 kcal/mol in case of Tyrosine kinase (Metformin -4.82 kcal/mol) with maximum number of targets along with some key interacting residues. Therefore, it can be proposed to have a potential role in ameliorating AD symptoms and can also be used in further research for treating Alzheimer's disease (Type-3 diabetes).

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