Characterization of putative drugs for targeting Alzheimer's disease and Type II Diabetes Mellitus

A Major Project Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of

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Submitted by

ALKA RAINA

(2K15/BIO/01)

Delhi Technological University, Delhi, India

Under the supervision of

Dr. Pravir Kumar

Associate Professor

Department of Biotechnology

Delhi Technological University

(Formerly Delhi College of Engineering)

CERTIFICATE

This is to certify that the dissertation entitled "**Characterization of putative drugs for targeting Alzheimer's disease and Type II diabetes"** submitted by **Alka Raina (2K15/BIO/01)** in the partial fulfilment of the requirements for the award the degree of Master of Technology (Bioinformatics), 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.

Date: 04 July 2017

Dr. Pravir Kumar
(Project Mentor)
Department of Bio-Technology
Delhi Technological University

Prof. D. Kumar
(HOD)
Department of Biotechnology
Delhi Technological University

DECLARATION

I, **Alka Raina,** do hereby declare that the dissertation entitled *"**Characterization of putative drugs for targeting Alzheimer's disease and Type II diabetes mellitus"*** has been undertaken by me for the award of Master of Technology in Bioinformatics. I have completed this study under the guidance of **Dr. Pravir Kumar**, Associate professor at Department of Biotechnology**,** Delhi Technological University, 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: 04 July 2017

Alka Raina

Registration No: 2K15/BIO/01

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Alka Raina
2K15/BIO/01

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
AICD	Amyloid precursor protein Intracellular Cytoplasmic/C-terminal domain
AβPP	Amyloid precursor protein
Aβ	Amyloid beta
BACE1	Beta site APP cleaving enzyme-1
IDE	Insulin degrading enzyme
NCBI	National Centre for Biotechnology Information
NEP	Neprilysin
NFTs	Neurofibrillary tangles
PDB	Protein Databank
PHFs	Paired helical filaments

Characterization of putative drugs for targeting Alzheimer's Disease and Type II Diabetes Mellitus

Alka Raina

Delhi Technological University, Delhi, India
E-mail ID: alkarainaj92@gmail.com

ABSTRACT

Alzheimer's disease (AD) is an incurable and debilitating neurodegenerative disease, characterized by amyloid-beta (Aβ) agglomeration and tau hyperphosphorylation which is further accountable for the formation of senile plaques thereby causing synaptic loss. Mounting evidences have revealed an interlink between AD Type II diabetes mellitus (T2DM). T2DM is an age-related disease characterised by hyperglycemia, hyperinsulinemia and insulin resistance (IR), along with the incapability of utilizing glucose as an energy currency. The strong association between AD and T2DM suggests a shared pathology like cognitive impairment, mitochondrial dysfunction, and reactive oxygen species (ROS) production and thus provide a platform to explore the therapeutic potential of proteins shared between the two diseases. Since, the two well-known major proteins namely, Insulin degrading enzyme (IDE) and Neprilysin (NEP) has reportedly been involved in the degradation of Aβ peptides and insulin respectively therefore, provides a substantial evidence for the connective link between AD and T2DM. Further, normal expression of these proteins in the neuron is beneficial for normal homeostasis of brain because of its peptidase activity. On the contrary, its higher expression causes severe damage to the cells thereby leading to the advancement of oncogenic processes. Thus

controlling the level of these proteins is quite necessary for maintaining the homeostatic balance of neuronal cells. In current work, we have attempted to investigate the important biomolecles like curcumin, apomorphine, nobiletin, resveratrol, and norepinephrine that could maintain or regulate the levels of IDE and NEP to decline the chances of rapid onset of AD and T2DM. These biomolecules have an inevitable role to escalate the levels of IDE and NEP and most importantly, in the clearance mechanism to degrade the Aβ plaques. For this purpose, we have employed various *in silico* methods such as drug-likeliness parameters namely Lipinski filter analysis, Ghose parameters, Veber rules, ADMET analysis, active site prediction and molecular docking studies. Finally, the present study outlines the novel potential of these biomolecules in regulating both the IDE and NEP expression to attenuate the neuronal loss.

INTRODUCTION

Alzheimer's disease (AD) is an incurable and debilitating neurodegenerative disorder which is manifested by accumulation of amyloid-beta (Aβ) and tau hyperphosphorylation, followed by the formation of neurofibrillary tangles (NFTs). Aβ deposition alters numerous signaling cascades including the insulin signaling pathway and glucose metabolism in the brain which renders the expression of insulin encoding genes altered and leads to insulin resistance [1]. Growing evidences have also reported that the diabetic condition can exacerbate not only Aβ accumulation but also tau phosphorylation induced cognitive impairment and elevation of calcium levels thereby intriguing phosphorylation of calcium-dependent kinases [2]. Further, Type II diabetes mellitus (T2DM), a chronic age-related disorder, is primarily characterized by hyperinsulinemia, hyperglycemia and insulin resistance (IR). T2DM also renders a patient's body incapable of utilizing glucose as an energy currency, thereby leading to the perturbance in lipid metabolism. [3]. The commonalities between AD and T2DM indicate a convincing link between them **(Fig. 1)**. The effect of T2DM increases the neuronal damage resulting in the onset of AD and hyperglycemia induced oxidative stress leading to the formation of reactive oxygen species (ROS). Additionally, the other mechanisms associated with T2DM encompass the polyol aldose reductase signaling activation accompanied with protein kinase C (PKC)-β triggering, dyslipidaemia, perturbed Na/K pump function and calcium balance. All the foregoing events have an impact on neuronal activity, mitochondrial function, membrane permeability, endothelial tissue and therefore expedite the cerebral aging by the process of peroxidation of polyunsaturated fatty acids, injure cell membrane integrity and induce apoptosis [4]. Importantly, involvement of Insulin degrading enzyme (IDE) and neprilysin (NEP) plays an inevitable role between AD and T2DM since these major enzymes are accountable for the degradation of Aβ. Conversely, under

certain physiological conditions, during aging, the activity of these enzymes get affected due to diminished expression or structural modification, which results in the reduction of Aβ clearance and its deposition in the brain. [5]. IDE is reported to degrade both insulin as well as Aβ and the inevitable role of IDE and NEP in the intra-cytosolic clearance of Aβ suggests that these could be therapeutically helpful for the treatment of AD [6]. Importantly, administration of different biomolecules may be helpful in regulating the altered levels of both IDE and NEP in the cells. Targeting these enzymes with numerous biomoleules viz, curcumin, apomorphine, nobiletin, resveratrol, and norepinephrine with intent to escalate their levels in the brain could be beneficial for treating both the diseases, AD and T2DM. Curcumin, a known flavonoid, elevates Aβ clearance by escalating the activity of both IDE and NEP and also prevents the production of Aβ by inhibiting presenilin 2 (PS2). Similarly, Apomorphine a dopamine receptor agonist promotes the degradation of Aβ via activation of IDE. Likewise, nobiletin enhances NEP activity both at the gene and protein level and thereby promotes Aβ clearance. Additionally, resveratrol also escalates NEP level that declines the Aβ deposition by upregulation of estradiol. Also, norepinephrine enhances the degradation of Aβ by upregulation of IDE [1]. These biomolecules are having chemoprotective, anti-inflammatory, neuroprotective and anti-aging property. For this purpose, we have performed *in silico* based structural and functional analysis of these molecules for revealing its therapeutic importance against neuronal loss via modulating the impaired expression of both IDE and NEP. The prime objective of this study is to explore neuroprotective role of these biomolecules in modulating the altered level of both IDE and NEP to attenuate the neuronal death.

LITERATURE REVIEW

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a most common age-related neurodegenerative disorder exhibiting the characteristics of Aβ accumulation, tau hyperphosphorylation and neurofibrillary tangles (NFT) formation. In addition to the intraneuronal formation of Aβ plaques and aggregation of tau protein, several other characteristics include neuroinflammation, mitochondrial dysfunction, oxidative stress and non-equilibrium between Aβ formation and its clearance mechanism. This perturbed equilibrium condition further leads to the abnormal burden of amyloid (Aβ) which eventually results in dementia and cognitive decline. AD depicts 70% of dementia cases as it has affected millions of people worldwide. AD is a complicated disorder, having destructive effects in terms of morbidity and mortality that is pathologically characterized by extracellular amyloid aggregates and tangles resulting in neuronal dysfunction. Current researches suggest mounting evidences for insulin and insulin-like growth factor resistance to be an another cause for promoting the etiology of AD. Since the predominant AD form is a sporadic having unsettled etiology and no certain treatment for curing the disease is available. Moreover, the prerequisite for AD drugs is to traverse through the blood brain barrier (BBB). The BBB is a dynamic barrier that has selective permeability feature, allows only selected biomolecules to enter, due to which administration of drugs to central nervous system (CNS) becomes strenuous [7]**.** The cleavage of the amyloid precursor protein (APP) by ϒ-secretase results in the production of many amyloid β (Aβ) types, including Aβ (40-43). The aggregation of Aβ into neurotoxic oligomers/ Aβ plaques leads to synaptic dysfunction and neuronal death [8]. In normal people, aggravated levels of amyloid in brain could be linked to the risk of developing AD later on or the Alzheimer-linked cognitive decline. The brain amyloid level in normal people when compared with the

exasperated levels depicted the higher chances of cognitive impairment [9]. Magnetic resonance imaging (MRI) can be used to evaluate the changes in brain, like the presence of amyloid β (Aβ) plaques, other functional and biochemical imbalance [10-14].

KEY GENES INVOLVED IN THE PROGRESSION OF AD

1. Abnormal hyperphosphorylation and oligomerization of Tau

Tau is the significant microtubule associated protein (MAP) of a developed neuron. The other two neuronal MAPs are MAP1 and MAP2. An important activity of MAPs is their association with tubulin and advancement of its gathering into microtubules and adjustment of the microtubule nexus. The microtubule gathering advancing action of tau, a phosphoprotein, is managed by its level of phosphorylation. Hyperphosphorylation of tau discourages this biological action of tau. In AD brain, tau is approximately three to four overlap more hyperphosphorylated than the normal tau and in this hyperphosphorylated condition, it is polymerized into paired helical fibers (PHF) along with straight fibers (SF), leading to the formation of neurofibrillary tangles (NFT) [15]. Tau is temporarily hyperphosphorylated amid improvement and amid anesthesia and hypothermia however not to an indistinguishable state from in AD brain. Some of the tau in AD is truncated which additionally advances its self-congregation [16]. Tau transformations found in frontotemporal dementia clearly advance its unusual hyperphosphorylation. In this manner, the unusually hyperphosphorylated tau is discernable from both typical and temporarily hyperphosphorylated taus, as it is inhibitory in a cytosolic/oligomeric state however not when it is self-congregated into PHF/SF. Inhibiting the irregular hyperphosphorylation of tau suggests a promising therapeutic focus for AD and related tauopathies [17]. Neurofibrillary degeneration of unusually hyperphosphorylated tau is found in

AD patients as well as in a group of related neurological disorders, tauopathies [18]. Till date, in AD as well as in each known human tauopathy, the tau pathology is comprised of the unusually hyperphosphorylated tau. In AD the majority of the six tau isoforms is hyperphosphorylated and congregated into PHF [19]. While conformational changes and truncation of tau resulting after its hyperphosphorylation have been accounted for in AD, the most settled and the most convincing reason for dysfunctioning of tau in AD and related tauopathies is its unusual hyperphosphorylation [20].

2. Amyloid precursor protein (APP) and Amyloid beta (Aβ) metabolism

The enzymatic procedures leading to the processing of APP to Aβ are currently known. APP is successively digested by two layer bound endoprotease exercises, β-and γ-secretase [21]. β-secretase initially separates APP to discharge a huge secreted product, sAβPP. A piece of 99 amino acids (CTFβ, which starts with the N-terminal aspartyl buildup of Aβ) remains layer bound, and is thus quickly separated by γ-secretase to produce Aβ [22]. Cleavage by γ-secretase is fairly uncertain, bringing about C-terminal heterogeneity of the subsequent peptide populace. Thus, various distinctive Aβ species exist, yet those closure at position 40 (Aβ40) are the most ample (~80-90%), trailed by 42 (Aβ42, ~5-10%). The somewhat longer types of Aβ, especially Aβ42, have higher hydrophobicity and fibrillogenicity, and are the central species aggregated in the brain [23, 24].

β-secretase action is accepted to be the rate constraining stride in the amyloidogenic pathway, and cleaves ~10% of the aggregate AβPP. The rest of the AβPP, nearly 90%, is constitutively cut by α-secretase creating sAPPα and CTFα [25]. Then, the γ-secretase cleavage of CTFα generates the more generous p3 piece rather than Aβ. γ-Secretase processing of membrane bound CTF likewise creates a cytosolic component, AICD which may act in signal transduction. As a result

of their basic part in the production of Aβ, both β-and γ-secretase are taken as prime focuses for the AD therapeutics [26]. Despite the fact that the measure of γ-secretase movement does not seem to increment in AD, adjustments in γ-secretase action prompting the creation of longer types of Aβ are the major hereditary reason for early onset, familial AD, an impact that can be imitated with an assortment of allosteric γ-secretase tweaking agents [27]. β-Secretase is an aspartyl protease, yet one that cuts APP and its different substrates outside of the bilayer [28]. There are two main types of BACE, BACE1 and BACE2, that are >65% homologous. The significant type of the enzyme leading to the formation of Aβ, BACE1, is exceedingly expressed in the brain. Conversely, the second type, BACE2, is in low levels in brain however is available in most tissues in elevated amounts. BACE1 is the main β-secretase, some remaining action may be owing to BACE2, and both types of BACE can go after substrate. β-Secretase action and protein are both immensely escalated in sporadic AD [29]. This impact demonstrates a local selectivity of brain that generally parallels ailment influenced regions and involves plaque formation and disorder span [30].

3. Presenelin 1 & 2 (PSN1&2)

Up until now, AD-causing mutations in PSEN1 that have been recognized are more than 150.around 10 extra mutations have been recognized in the PSEN2 and 25 changes have been distinguished in the APP gene [31]. The understanding of PSEN1 is hence essential for discovering the pathogenesis of familial AD. Major mutations in PSEN1 are basic missense ones that leads to single amino-acid change in presenilin1 [32]. Some are more complicated, like deletions, inclusions. The most serious mutation in PSEN1 is a donor–acceptor transformation that causes two AA substitutions and a frame deletion of exon 9 [33]. Fundamentally, the biochemical outcomes of these changes for γ-secretase congregations are limited. Mutations in

either presenilin or APP reliably increment the relative proportion between the long ($A\beta_{42}$) and short ($A\beta_{40}$) amyloids ($A\beta_{42}/A\beta_{40}$).since the inactivation of Psen1 and Psen2 totally inhibits Aβ production, this expansion can without a doubt be considered as a toxic function gain [34]. Although, the adjustment in proportion can likewise be the result of a halfway loss of $A\beta_{40}$ production [35]. However, the research so far has depicted that presenilin 1 is critical for keeping up the normal functioning of the brain; it is less evident whether the extreme deficiencies in homozygous loss-of-capacity mouse models are significant to the pathology in human patients. Besides, PSEN1 lack is probably not going to add to the disorder in patients with APP changes, which suggests that the impacts of loss of PSEN1 capacity on APP preparing are critical for our comprehension of the pathogenesis of AD [36].

DIABETES

Diabetes mellitus, a gathering of metabolic disorders, has the characteristics of hyperglycemia coming about because of deformities in insulin secretion, insulin activity, or both [37]. The interminable hyperglycemia of diabetes is related with damage, and dysfuntioning of different organs, particularly the eyes, kidneys, nerves, heart, and veins. A few pathogenic procedures are included in the improvement of diabetes [38]. These range from immune system devastation of the β-cells of the pancreas with ensuing deficiency of insulin to irregularities that lead to imperviousness to insulin activity [39]. The premise of the variations from the norm in sugar, fat, and protein digestion in diabetes is inadequate activity of insulin on the target tissues. Lacking insulin activity comes about because of insufficient insulin discharge or potentially reduced tissue reactions to insulin at least one focuses in the complex pathways of hormone activity [40]. Hindrance of insulin discharge and imperfections in insulin activity often exist together in a similar patient, and it is regularly indistinct which variation from the norm, if either alone, is the

essential driver of the hyperglycemia [41]. Side effects of stamped hyperglycemia incorporate polyuria, polydipsia, weight reduction, once in a while with polyphagia, and obscured vision. Disability of development and being susceptible to specific diseases may likewise go with interminable hyperglycemia. Intense, life-undermining outcomes of non-controllable diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar disorder [42].

Types of diabetes

1. Type I diabetes mellitus (T1DM)

The type of diabetes, which represents just 5–10% of diabetic population, incorporated by the terms like type I diabetes, insulin-dependent diabetes, or adolescent onset diabetes, comes about because of an autoimmune devastation of β-cells that is cell-mediated [43]. In this type of diabetes, the β-cell demolition rate is very different ranging from being fast in a few people (chiefly babies and kids) to moderate in others (basically grown-ups). A few patients, especially youngsters and youths, may give ketoacidosis as the main sign of the disorder. While others are having unassuming fasting hyperglycemia that can quickly change to serious hyperglycemia and additionally ketoacidosis within the sight of disease or different stressful conditions [44]. Adults may have leftover β-cell working adequate to avoid ketoacidosis for a long time; such people in the long run become subjected to insulin for survival and are at hazard for ketoacidosis. At this last phase of the illness, there is practically zero insulin secretion. Type I diabetes regularly happens in youth and pre-adulthood, yet it can happen at any age. Immune system decimation of β-cells has various genetic predispositions and is likewise linked to natural factors that are still inadequately characterized. Despite the fact that patients are once in a while plump when having this kind of diabetes, being obese is not inconsistent with analysis [45].

2. Type 2 diabetes mellitus (T2DM)

Type II diabetes represents ~90–95% of diabetic population and is already alluded to as non-insulin-independent diabetes. It includes people having insulin resistance and typically relative (instead of total) insulin inadequacy. At first, and frequently all through their lifetime, these people, for their survival, need not bother with insulin treatment. Various reasons for this type of diabetes are presumably present [46]. In spite of the fact that the particular etiologies are unknown, obliteration of β-cells does not take place. Most patients with this type of diabetes are having obesity, and it causes some level of insulin resistance. Patients who are not fat by weight aspect may have an expanded rate of muscle to fat quotients disseminated overwhelmingly in the stomach locale. Ketoacidosis from time to time happens suddenly in this kind of diabetes [47]. This type of diabetes usually goes undiscovered for a long time in light of the fact that the hyperglycemia grows bit by bit and in initial stages, it is regularly not sufficiently extreme for the patient to see any of the exemplary manifestations of diabetes. These patients are at expanded danger of having macrovascular and microvascular difficulties. Though patients having type of diabetes may have insulin levels that seem ordinary or escalated, the higher blood sugar level would be relied upon to bring about significantly higher insulin. In this way, insulin discharge is faulty in these patients and lacking to make up for insulin resistance. Insulin resistance may get better with weight loss or potentially pharmacological treatment of hyperglycemic, yet is rarely reestablished to typical. The danger of building up such type of diabetes increments with age, stoutness, and absence of physical movement. It is more common in women and in people having hypertension or dyslipidemia. It is regularly connected with solid genetic predispositions. In any case, the hereditary qualities of this type of diabetes are intricate and not obviously characterized [48].

INTERLINK BETWEEN AD AND T2DM

Factors, for instance, "Insulin, insulin receptor (IR),and insulin–like growth factor-1 (IGF-1)" has an important part to play in brain by means of managing metabolism of brain, neuronal development, and process of differentiation. Any objection in the cross-talk amongst insulin and neuronal glucose digestion may retard the ATP production that results in apoptosis of neurons. This contexts with the non-functionality of metabolism of energy connected with stress-incited changes in the IR signaling cascade and the parallel demise signals that lead to apoptosis [49]. Mounting indisputable confirmation revealed that diabetes and modified insulin signals may have a significant effect on development of AD. Diabetes mellitus is a standout amongst the most metabolic issue, basically represented by impedance in insulin signaling with pervasiveness in matured people. Additionally, modifications of cognitive abilities have been expressed in both T1DM and T2DM patients [50]. The cardinal imperfection in T2DM is insulin resistance, whose roots are deficiency of insulin, and falls under metabolic and vascular damage factors, namely "metabolic disorder" so this scenario specifically relates insulin to dementia and cognitive impairment in T2DM. Besides, under dementia-sort of issue, T2DM has been progressively connected to AD. The associations between T2DM and AD encompasses elevated cholesterol levels, age related disorders, CNS and PNS insulin resistance, non-functional insulin receptors (IR) and IR signaling, diminished glucose transport, and neuronal degeneration. Subsequently hyperinsulinemia/hypoglycemia in T2DM, cognitive decline advances AD onset. Also, a few studies of the disease revealed that T2DM escalates the chances of AD, and it is conceivable that hyperinsulinemia has a conclusive part in AD development.

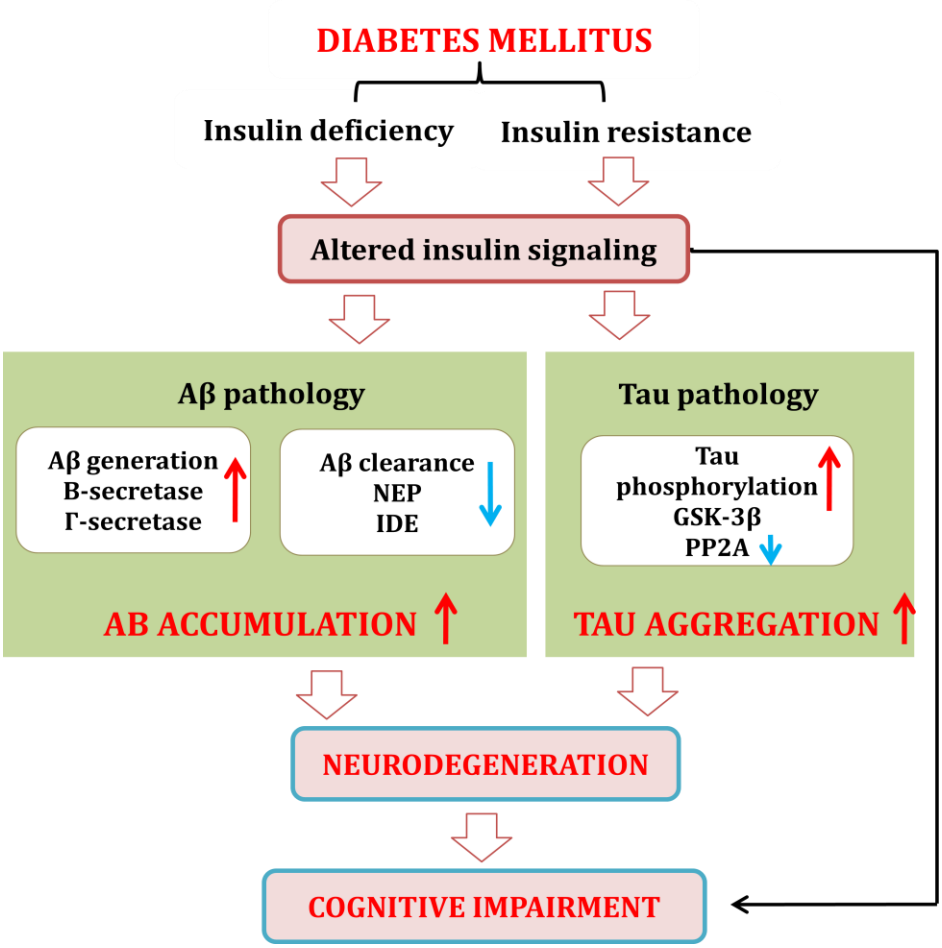


Figure 1: Interlink between T2DM and AD

INSULIN DEGRADING ENZYME (IDE)

Insulin-degrading enzyme (IDE or insulysin), approximately 110-kDa thiol zinc-metalloendopeptidase, is situated in the cellular parts like endosomes, peroxisomes, cytosol and on the surface of cell that divides proteins having differing amino acid sequences, huge numbers of which frame β-pleated sheet-rich amyloid fibrils under specific conditions [51]. The significant enzyme responsible for degradation of insulin is IDE *in vitro*, yet the degree to which it intervenes insulin catabolism in vivo has been disputable. Insulin, which is basic for glucose, lipid, and protein digestion, in addition for cell development and separation, is cleared basically

by the liver and kidney, however most different tissues likewise debase the hormone. Besides having a putative part in catabolism of insulin, reports suggests that IDE degrades Aβ in neuronal and microglial cells, and to dispose of Aβ's neurotoxic impacts. however, cerebral aggregation of Aβ is accepted to give its role in the pathogenesis of AD, so far in most of the cases, the fundamental reasons for this rise are obscure. A few lines of confirmation exhibit that recently created Aβ is quickly cleared, proposing that proteases degrading Aβ could assume a part in managing cerebral levels of this peptide. Neprilysin (NEP) and endothelin-changing over catalyst have been appeared for the degradation of Aβ, in vivo and approving the part of proteolysis in modulating endogenous Aβ levels. Moreover, with its hereditary linkage to DM2, the IDE part of chromosome 10q has likewise been connected to late-onset AD and to time of disorder onset in AD families [52]. Allelic relationship in or close to the IDE quality has been accounted for in some AD populaces yet not others. In two different reports, the event of AD and high plasma Aβ42 levels were connected to a part of chromosome (10q) which is approximately 40Mb proximal to the linkage crests over the IDE. It has latently been established, in cell cytosol divisions that IDE carries out the degradation of β-amyloid precursor protein (APP), intracellular area (AICD), which can generally achieve the nucleus and take an interest in transcriptional modulation.

NEPRILYSIN (NEP)

The degradation of Aβ by proteases is the main source of clearance mechanism. A wide range of such proteases have been recognized and this matter has been extensively studies. Among these proteases, neprilysin (NEP) is a standout as it is the most imperative for the control of the levels of cerebral Aβ [53]. This enzyme, approx. 97 kD and cell surface-bound, acts in the peripheral and central nervous system (CNS) where it apparently degrades the peptides. Utilizing the

radiolableled Aβ, Iwata et al. demonstrated that Aβ42 was primarily degraded by NEP in their in vivo experiment. Moreover, use of NEP inhibitors in rodent brain created sensational rises of endogenous Aβ bringing about plaque formation. This impact was separately recreated in mice. Additionally supporting NEP to be the basic Aβ-degrading protease is the perception that NEP over expression confers drastic decreases in Aβ plaque formation in APP-transgenic mice, and in a few tests, enhanced cognitive abilities. It has additionally been demonstrated that NEP, mRNA and protein expression levels are diminished in relationship with age or in AD cases. An exceptionally particular compound immunocapture/movement test to demonstrate that NEP action levels increment with age and amid the movement of AD has been utilized [54]. This is like the agreement on most AD related endopeptidase expression levels, and may depict a homeostatic reaction to the huge amounts of Aβ substrate and additionally to the fiery condition happening in AD. In any case, these expanded endogenous levels of enzymes degrading Aβ are at last deficient to keep the aggregation of Aβ in AD. Despite the information showing the significance of NEP in degradation of Aβ, different compounds are deserving of clinical review. For instance, NEP knockout mice indicate just a direct (1.5–2 overlay) increment in Aβ levels that are a long way from the levels expected to incite plaque formation, as seen with NEP inhibitors. This unassuming increment in Aβ increases the likelihood of other Aβ degrading proteases that are in like manner responsive to NEP-inhibitors, alike NEP [55].

SIGNALING MECHANISMS

The potential medication target in the treatment of T2DM is an insulin degrading enzyme (IDE). In muscle tissues, the insulin circulatory level is regulated by IDE via a degradation based clearance mechanism [56]. Besides IDE, clearance of Aβ is activated through NEP that follows up on various targets that is what leads to Aβ aggregation. Observations suggest that over expressing IDE degrades AICD and insulin to fundamentally decreased Aβ plaques in brain [57]. Moreover, AICD binding to the promoter of NEP prompts transcriptional activation of NEP by aggressive supplanting with histone deacetylases (HDACs). This transcriptional activation leads to an increased expression of NEP as well as Aβ clearing [58]. As of late, it has been discovered that IDE's long isoform (IDE-Met1) is likewise included in Aβ degradation after the mitochondrial biogenesis pathway. The mitochondrial biogenesis pathway is connected with mitAβ levels and organelle usefulness by IDE-Met1. Initiation of PGC1-α by the impact of mitochondrial biogenesis jolts advance expression of NRF-1, that yields long isoform (IDE-Met1) and the short isoform (IDE-Met42) of IDE [59]. Moreover, the long isoform of IDE is included in the mitochondrial Aβ degradation with no lethal impact. For Aβ degradation, another contender is ApoE that works inside microglia and in the extracellular space to influence the Aβ clearance via advancing proteolysis of Aβ by IDE and NEP [60]. Aβ's degradation (endolysis) inside microglia is encouraged by NEP, while extracellular degradation is carried out by IDE. Since, ApoE is capable to advance degradation of Aβ and this relies upon the isoform of ApoE and the lipidation status; hence, lipidated ApoE is the resultant of transference of lipids to ApoE, which is proficient essentially by and LXR and ABCA1 [61]. It has additionally been accounted for that isoform of ApoE, ApoE4 is related with higher danger of AD, while the ApoE2 and ApoE3 isoforms is related with lower danger of AD contrasted and ApoE4. Furthermore, The

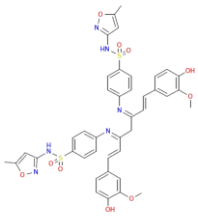
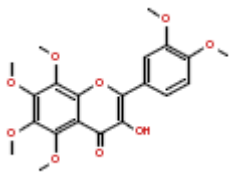
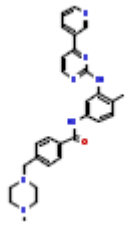
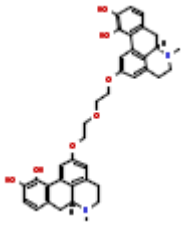
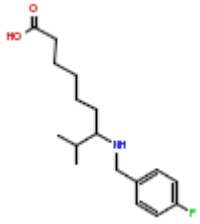
receptor for the propelled glycation final results (RAGE) go about as an Aβ transporter over the Blood-Brain Barrier (BBB) into the CNS where it stores Aβ, whereas the low-density lipoprotein receptor-related protein (LRP) intervenes additional Aβ burden outside the brain [62]. Both IDE and NEP are likewise required in RAGE interceded Aβ clearance, whereas, ApoE, ApoJ, and α 2 - macroglobulin are included in Aβ transportation outside the brain into the liver for degradation through LRP complex. Since, RAGE aids Aβ aggregation within the neuron therefore, IDE and NEP tie to RAGE and hinders their capacities so as to keep up memory discernment and neuronal survival [63].

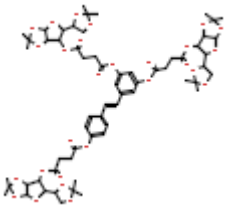
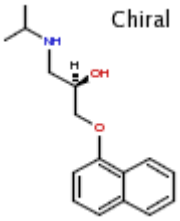
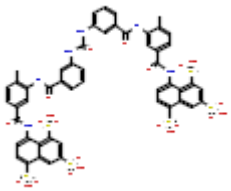
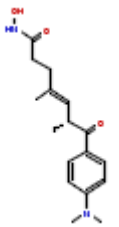
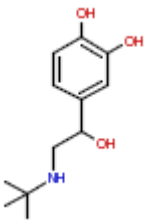
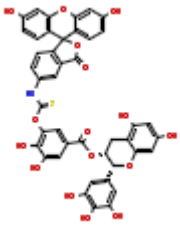
BIOMOLECULES

Biomolecules having neuroprotective effect against neurodegeneration are of tremendous importance so that the toxicity would be minimal and the effect would be higher. Administration of such biomolecules may be helpful in regulating the levels of essential proteins in the body. Importantly, administration of different biomolecules may be helpful in regulating the altered levels of both IDE and NEP in the cells. Targeting these enzymes with numerous biomoleules viz, curcumin, apomorphine, nobiletin, resveratrol, and norepinephrine with intent to escalate their levels in the brain could be beneficial for treating both the diseases, AD and T2DM. Curcumin, a known flavonoid, elevates Aβ clearance by escalating the activity of both IDE and NEP and also prevents the production of Aβ by inhibiting presenilin 2 (PS2). Similarly, Apomorphine a dopamine receptor agonist promotes the degradation of Aβ via activation of IDE. Likewise, Nobiletin enhances NEP activity both at the gene and protein level and thereby promotes Aβ clearance. Additionally, Resveratrol also escalates NEP level that declines the Aβ deposition by upregulation of estradiol. Also, Norepinephrine enhances the degradation of Aβ by

upregulation of IDE [1]. These biomolecules are having chemoprotective, anti-inflammatory, neuroprotective and anti-aging property.

Table 1: Pharmacokinetic properties of biomolecules

Biomolecules	Molecular structure	Physical properties	Action mechanism	Toxicity	Other Advantages
Curcumin		Mol. Formula:$C_{21}H_{20}O_6$ Mol. Mass: 372 Solubility: moderately soluble pH:2.5-7	Escalates Aβ clearance process by activating both IDE & NEP and inhibits presenilin 2 (PS2).	At higher conc., Gastrointestinal symptoms, nausea, diarrhea.	Enzyme inhibitors, anti-inflammatory, anti-neoplastic, anti-oxidant.
Nobiletin		Mol. Formula:$C_{21}H_{22}O_8$ Mol. Mass:402.39 Solubility: poorly soluble pH:4	Enhances NEP activity and promotes Aβ clearance.	—	Anti-angiogenic, anti-tumor, anti-inflammatory.
Imatinib		Mol. Formula:$C_{29}H_{31}N_7O$ Mol. Mass:493.259 Solubility:soluble pH:5.8	Tyrosine kinase inhibitor that increases the expression of NEP.	Hepatotoxicity, odema, nausea, vomiting, muscle cramps & fatigue.	Anti-carcinogenic, used for treating chronic myelogenic leukemia(CML), gastrointestinal stromal tumours (GISTs)& other malignancies.
Apomorphine		Mol. Formula:$C_{17}H_{17}NO_2$ Mol. Mass:267.126 Solubility: moderately soluble pH:5-6	A dopamine receptor agonist that promotes the intracellular Aβ degradation via activating IDE.	Heptatoxicity in rare cases.	Emetics, dopamine agonists.
Leptin		Mol. Formula:$C_{87}H_{138}N_{22}O_{28}S_2$ Mol. Mass:2002.949 Solubility:soluble pH:5-8	Enhances expression level of IDE by activating Akt pathway.		Increases arterial pressure, heart rate through central neural mechanisms and prevents the depressor effects of weight loss.

Resveratrol		Mol. Formula: $C_{14}H_{12}O_3$ Mol. Mass:228.079 Solubility: soluble pH:9	Increases the NEP level that decreases the Aβ accumulation.	Heptatoxicity, in rare cases.	Anti-inflammatory,anti-carcinogenic,anti-oxidant,anti-neoplastic,platelet aggregation inhibitors,anti-mutagenic.
Propanolol		Mol. Formula:$C_{16}H_{22}ClNO_2$ Mol. Mass:259.34 Solubility:soluble pH:2-3	Elevates the expression of IDE.	Heptatoxicity, bradycardia, cardiac failure, hypotension.	Anti-hypertensive,anti-arrythmia,vasodilator, adrenergic-beta antagonist.
Suramin		Mol. Formula:$C_{51}H_{40}N_6O_{23}S_6$ Mol. Mass:1297.29 Solubility:soluble pH:7	Enhances the activity of IDE by altering the turnover rate of the enzyme for its substrate.	___	Anti-neoplastic, anti-nematodal agents.
Trichostatin A		Mol. Formula:$C_{27}H_{42}O$ Mol. Mass:302.37 Solubility:soluble pH:4	Significantly enhances the NEP expression.	Low	Anti-tumor agents,Anti-fungal agents, anti-bacterial agents, histone deacetylase inhibitors.
Norepinephrine		Mol. Formula:$C_{22}H_{18}O_{11}$ Mol. Mass:176 Solubility:soluble pH:3-4	Stimulates the degradation of Aβ peptides by upregulation of IDE.	Low	Sympathomimetics, adrenergic-alpha agonist, vasoconstrictor agents.
EGCG		Mol. Formula:$C_{22}H_{18}O_{11}$ Mol. Mass:458.37 Solubility:soluble pH:4-6	Increases NEP activity thereby promotes Aβ degradation.	Heptatoxicity.	Anti-carcinogenic, anti-oxidant, anti-mutagenic agents.

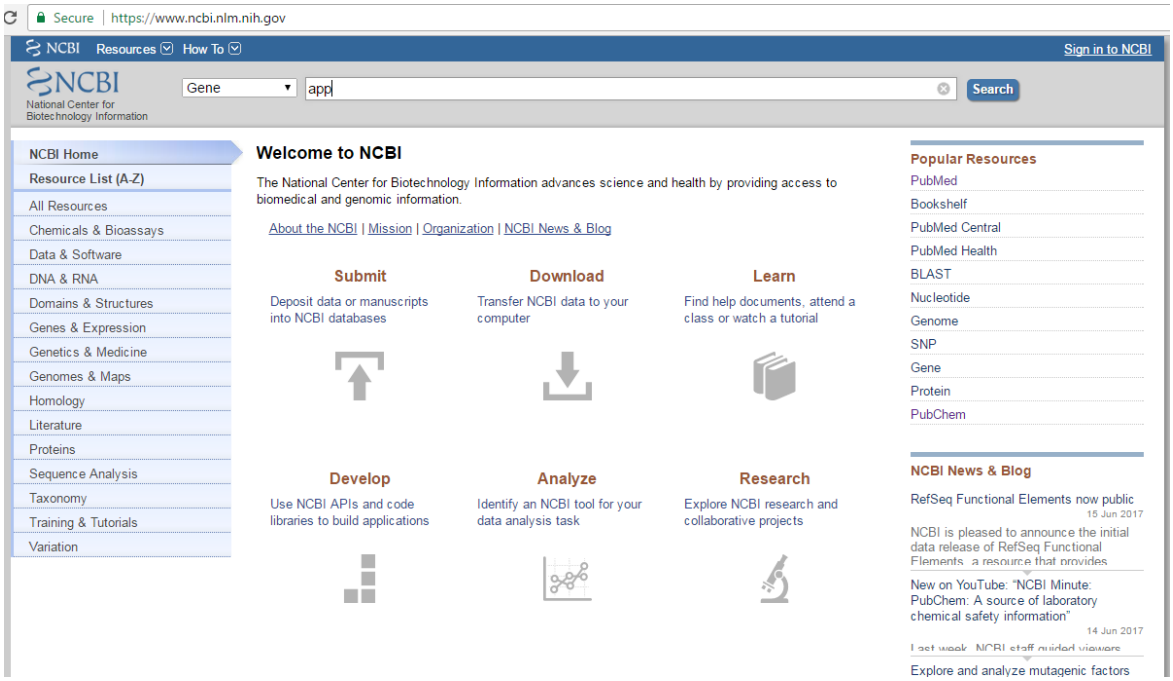
MATERIALS AND METHODS

Based on the review of literature, we were able to deduce the main link between the two age-related disorders. Mounting evidences suggest that there are two key proteins viz Insulin degrading enzyme (IDE) and Neprilysin (NEP) that could be targeted for treating both Alzheimer's disease (AD) and Type II Diabetes mellitus (T2DM). Regulating the levels of these two critical proteins is very important since their altered levels could result in the development of both AD and T2DM. So finding out the drug candidates (biomolecules) that could trigger the key proteins involved in AD and T2DM could be of an added advantage as it would be able to treat both the diseases simultaneously. Following are the methods used to deduce the common link between AD and T2DM, subsequently test the interaction of the selected biomolecules with the proteins and their efficacy.

RETRIEVAL OF GENE SEQUENCES AND PROTEIN STRUCTURES FROM THEIR RESPECTIVE DATABASES AND THEIR VISUALIZATION:

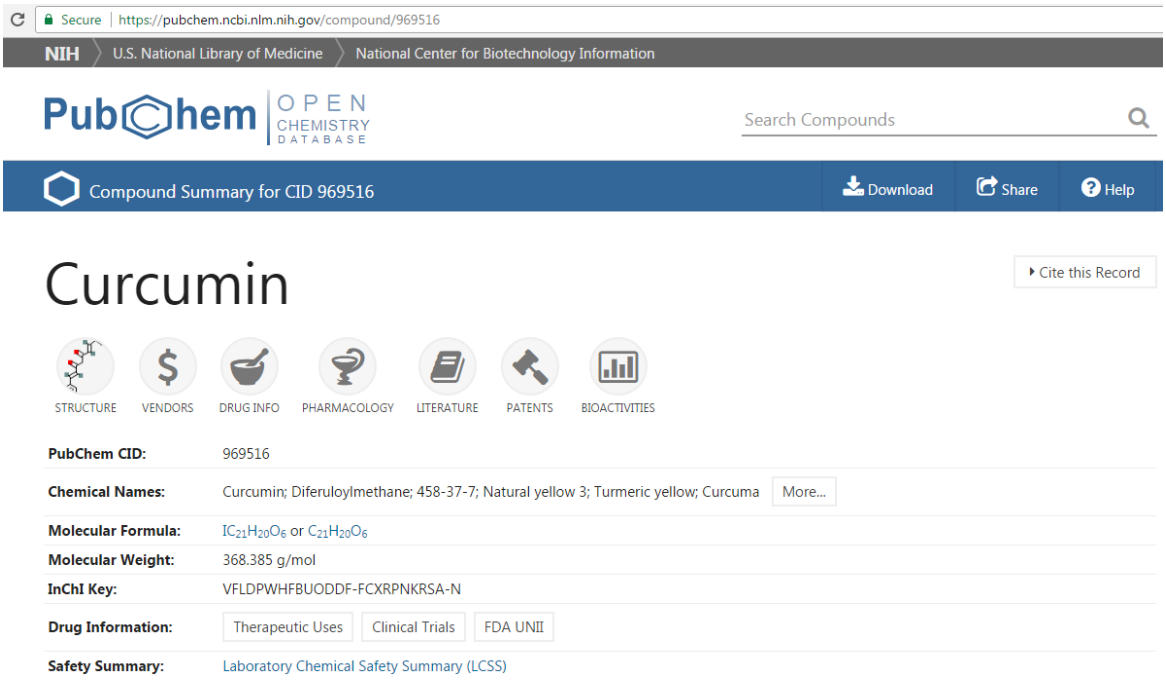
1. National centre for biotechnology information (NCBI)

The national centre for biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/) houses a progression of databases significant to biotechnology and biomedicine and is an imperative asset for bioinformatics instruments and administrations. Significant databases incorporate GenBank for DNA arrangements and PubMed, a bibliographic database for the biomedical writing. The gene sequences of AβPP, TAU, INS were retrieved from NCBI to carry out the multiple sequence alignment (MSA) so as to find out the conserved regions between the three genes which forms the basis for being susceptible to age-related disorders, AD and T2DM.



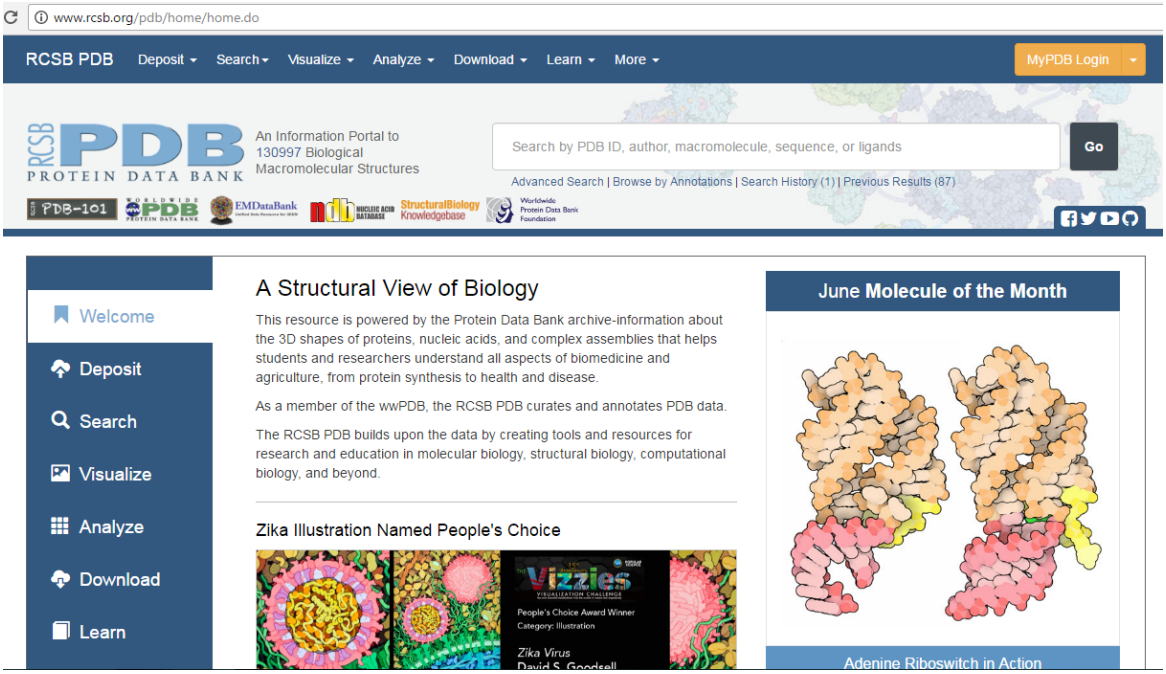
2. PUBCHEM

PubChem (https://pubchem.ncbi.nlm.nih.gov/) is an open-access archive comprising of an arrangement of three essential open databases viz compound, BioAssay, and Substance. It comprises data on an expansive scope of substance elements. PubChem comprises >150 million researcher-deposited compound informations, 60 million remarkable compound structures. All the biomolecules' molecular structures and their salient features were retrieved from PubChem. PubChem offers the structure file in sdf or mol format which needs to be optimized using a tool called Open babel tool. This tool converts the sdf or mol formats of molecules in to the required format; let's say pdb which was required here.



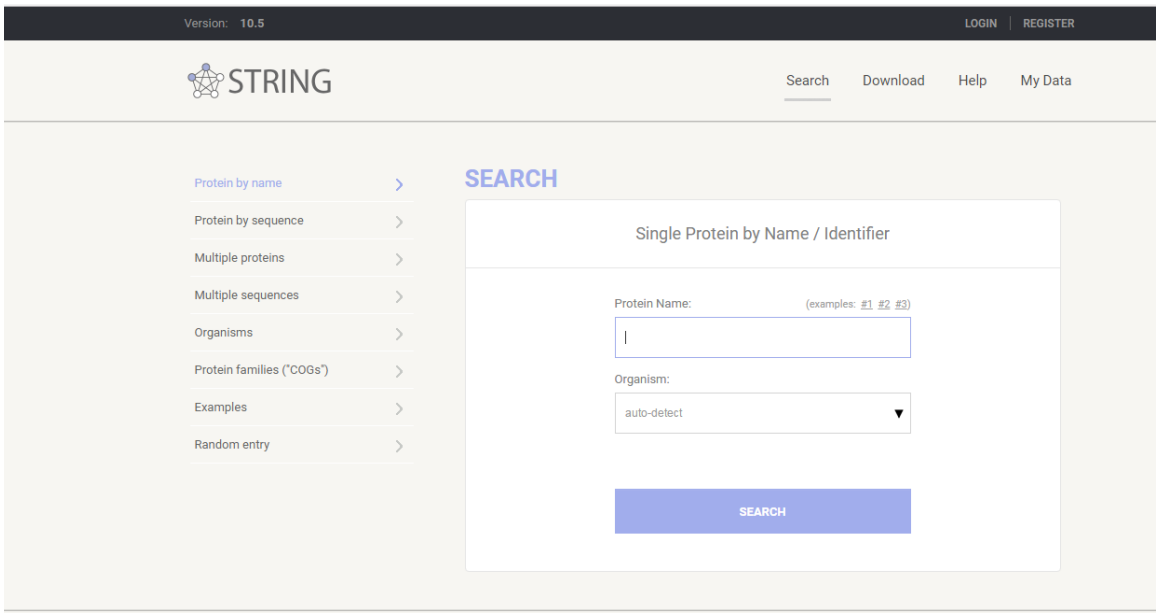
3. Protein data bank (PDB) database

The PDB document houses data for radically explored structures of complex assemblies, nucleic acids, and proteins. PDB represents a crystallographic database for 3D structural information of extensive organic compounds like nucleic acids and proteins. The information, ordinarily acquired by various techniques such as X-ray crystallography, NMR spectroscopy, is presented by researcher and chemists from around the globe and is made openly available on the Internet by means of the sites of its association partners. The structures of the proteins (IDE and NEP) were retrieved from PDB in the pdb format for further investigation of interactions and the structures of AβPP, TAU and INS were also retrieved from PDB for the protein-protein interaction study.



4. STRING DATABASE

STRING (Search Tool for the Retrieval of Interacting Proteins), a biological database, (https://string-db.org/) is an online asset for known and anticipated protein–protein interactions.STRING comprises of data from various sources like experimentally determined, computational estimation techniques and text accumulations from public. It has an open access and is routinely refreshed. String database is used to obtain the protein-protein interaction network. From the PPI network we could conclude that AβPP, INS & TAU (MAPT) are connected and APP has several other interacting partners viz MAPK8, BACE1, PSEN1, CASP6, SNCA as well which is the basis for the study to find a connecting link between AD and T2DM.

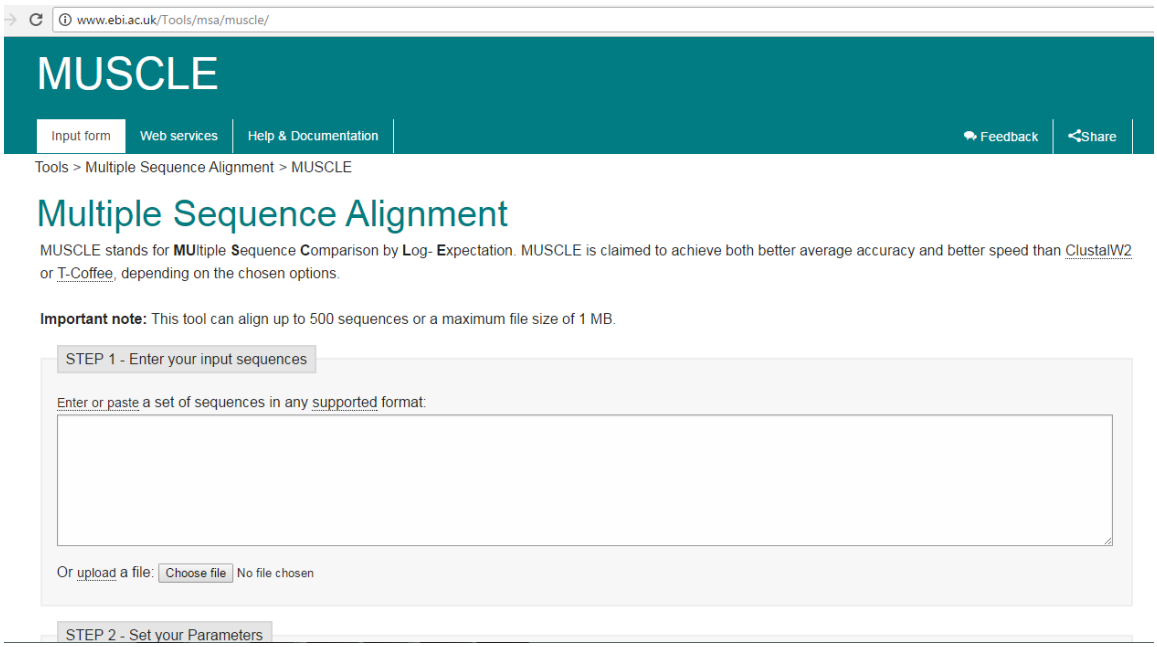


5. PYMOL

PYMOL, a 3D visualization tool, is used to visualize the three-dimensional (3D) structures of proteins, IDE and NEP and to locate their ligand binding site.

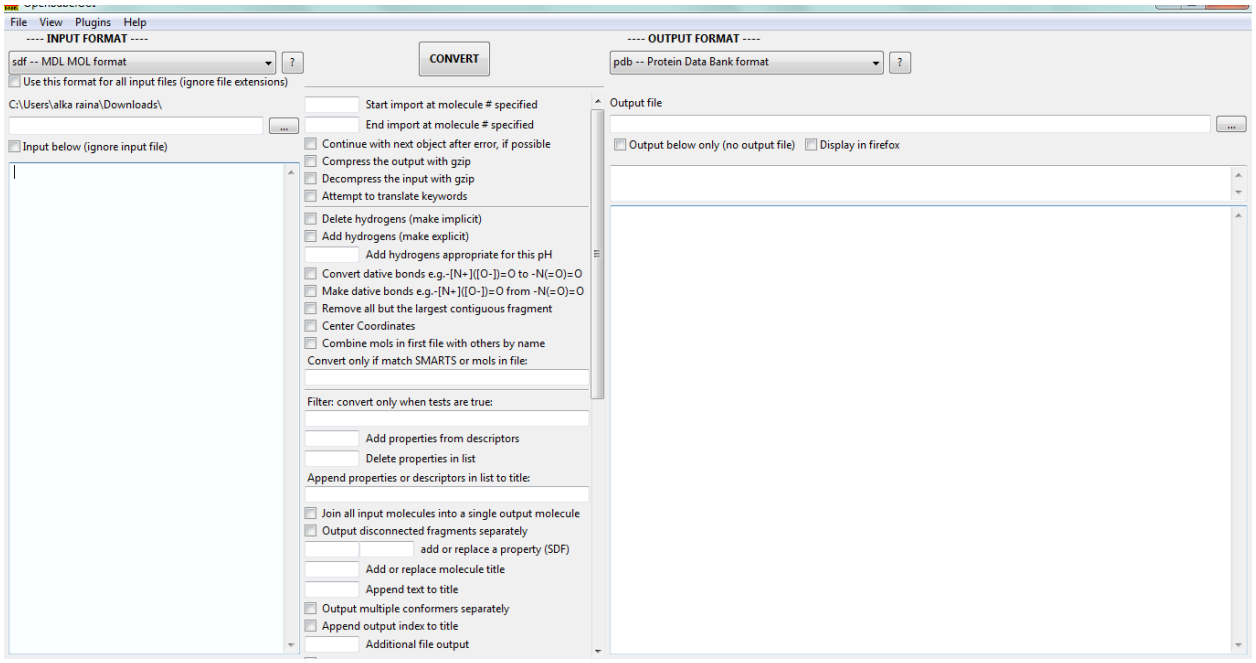
6. MUSCLE TOOL

For multiple sequence analysis of AβPP, INSULIN and TAU, Muscle tool (http://www.ebi.ac.uk/Tools/msa/muscle/) was used and phylogenetic tree was constructed based on NJ (Neighbor joining) plot without distance correction. The nucleotide sequences of the genes were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) for the multiple sequence alignment.



7. Optimization of ligand-protein structure

The protein structures of IDE and NEP were retrieved in the pdb format from Protein data bank (PDB) (http://www.rcsb.org/pdb/home/home.do). The ligand structures were retrieved in sdf format from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and they were converted into the pdb format using the open babel tool, as the docking requires uploading the protein and ligand structures both in the pdb format.



8. Lipinski filter analysis of screened drugs

An online tool Lipinski Filter (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) was used to retrieve the information about drug likeliness properties of Biomolecules based on Lipinski rule of five. Lipinski rule helps to differentiate between drug and non-drug like properties of molecules. It is used to identify the possibility of success or failure due to drug likeliness for molecules fulfilling with two or more of the following rules: (a) Molecular Mass should be less than 500 Dalton, (b) High Lipophilicity (expressed as logP less than 5), (c) Less than 5 hydrogen bond donors, (d) Less than 10 hydrogen bond acceptors, and (e) Molar refractivity should be between 40-130.

9. ADMET Analysis

ADMET stands for Absorption, Distribution, metabolism, Excretion and Toxicity. Predicting the ADMET properties plays an inevitable role in the process of drug design as these properties are accountable for the failure of about 60% of all drugs in the clinical trials. Traditionally ADME

tools were applied at the end of the drug development pipeline, but presently ADME is applied at an initial phase of the drug development process, in order to discard molecules with poor ADME properties from the drug development pipeline and this leads to remarkable savings in research and development costs. Swiss ADME tool (www.swissadme.ch/index.php) was used to check the toxicity profile of all the biomolecules. This Swiss ADME assesses various important parameters of ligands such as GI permeability, BBB permeability, bioavailability score etc.

10. Active site prediction

The pock drug tool was used to predict the active sites of both IDE and NEP (http://pockdrug.rpbs.univ-paris-diderot.fr/cgi-bin/index.py?page=home). Both IDE and NEP PDB structures were uploaded in the online tool and active sites were predicted using f-pocket estimation and setting ligand proximity threshold at 5.5.

11. Molecular docking

The optimized proteins and ligands were uploaded for molecular docking studies using the online tool namely, Docking Server (http://www.dockingserver.com/web). Kollman united atom type charges, essential H_2 atoms and solvation parameters were added using the Autodock tool. Affinity grid maps were generated with 0.375 Å spacing. Further, the van der Waals and electrostatic interactions were calculated using Autodock parameter set and distance-dependent dielectric functions respectively. The particular chains of IDE and NEP were selected for docking. The protein and ligand charges were calculated using gasteiger method with pH set to 7.0.

RESULTS

Phylogenetic relationship

For multiple sequence analysis, Muscle tool was used and found that amino acid residues were conserved in most of the isoforms of AβPP, Insulin and Tau protein **(Figure 2A)**. Phylogenetic study of these proteins revealed that these proteins were in same cluster as they share same homology. **(Figure 2B)**

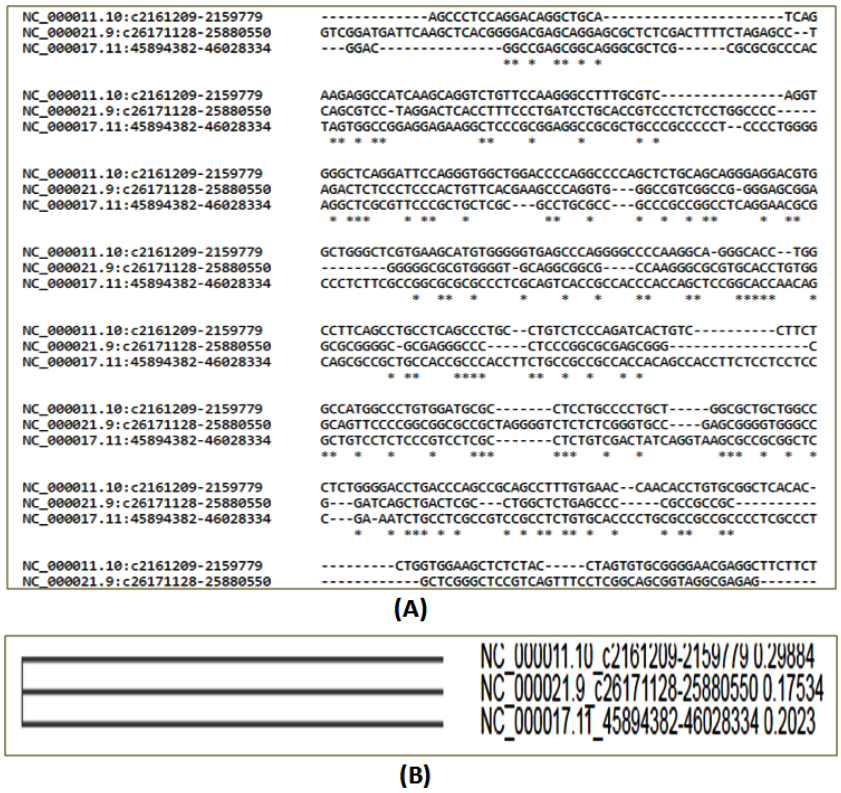


Figure 2: (A) Multiple Sequence Alignment and (B) phylogenetic Analysis of AβPP, Insulin and Tau protein

Pymol, 3D visualization:

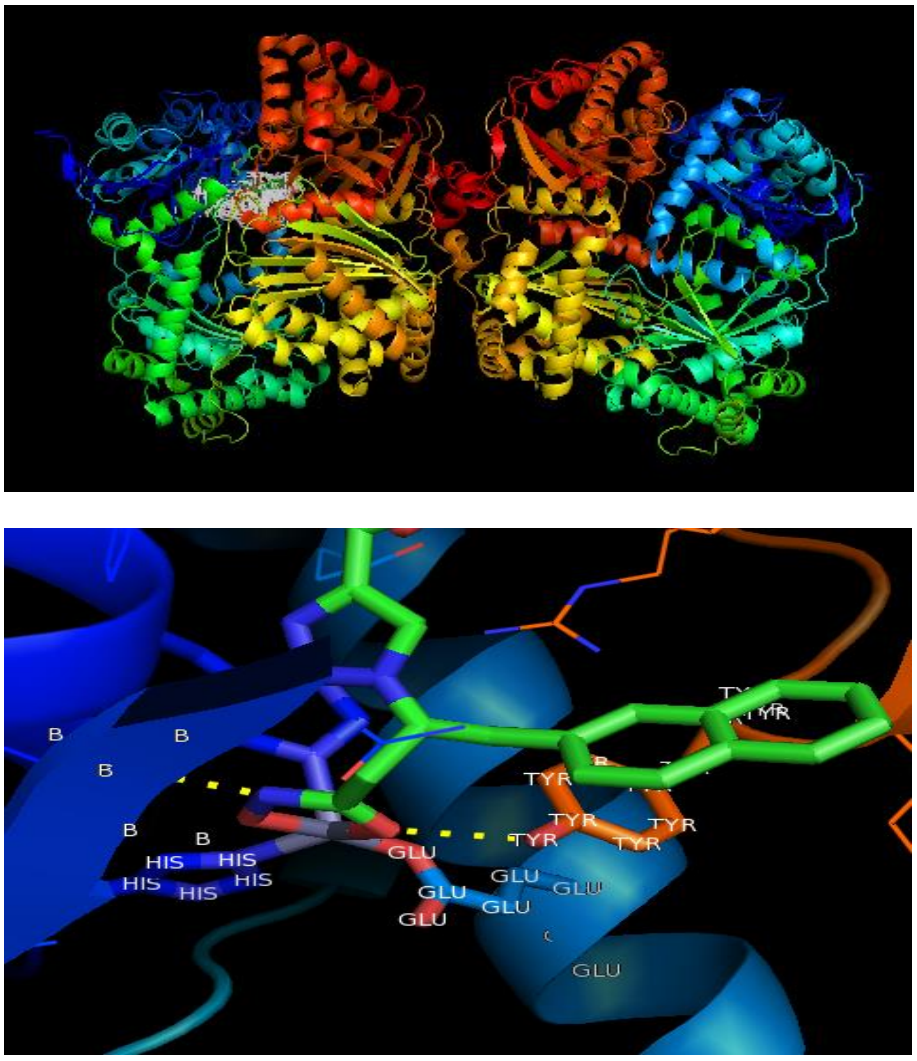


Figure 3: 3D Visualisation of insulin degrading enzyme (IDE) using Pymol

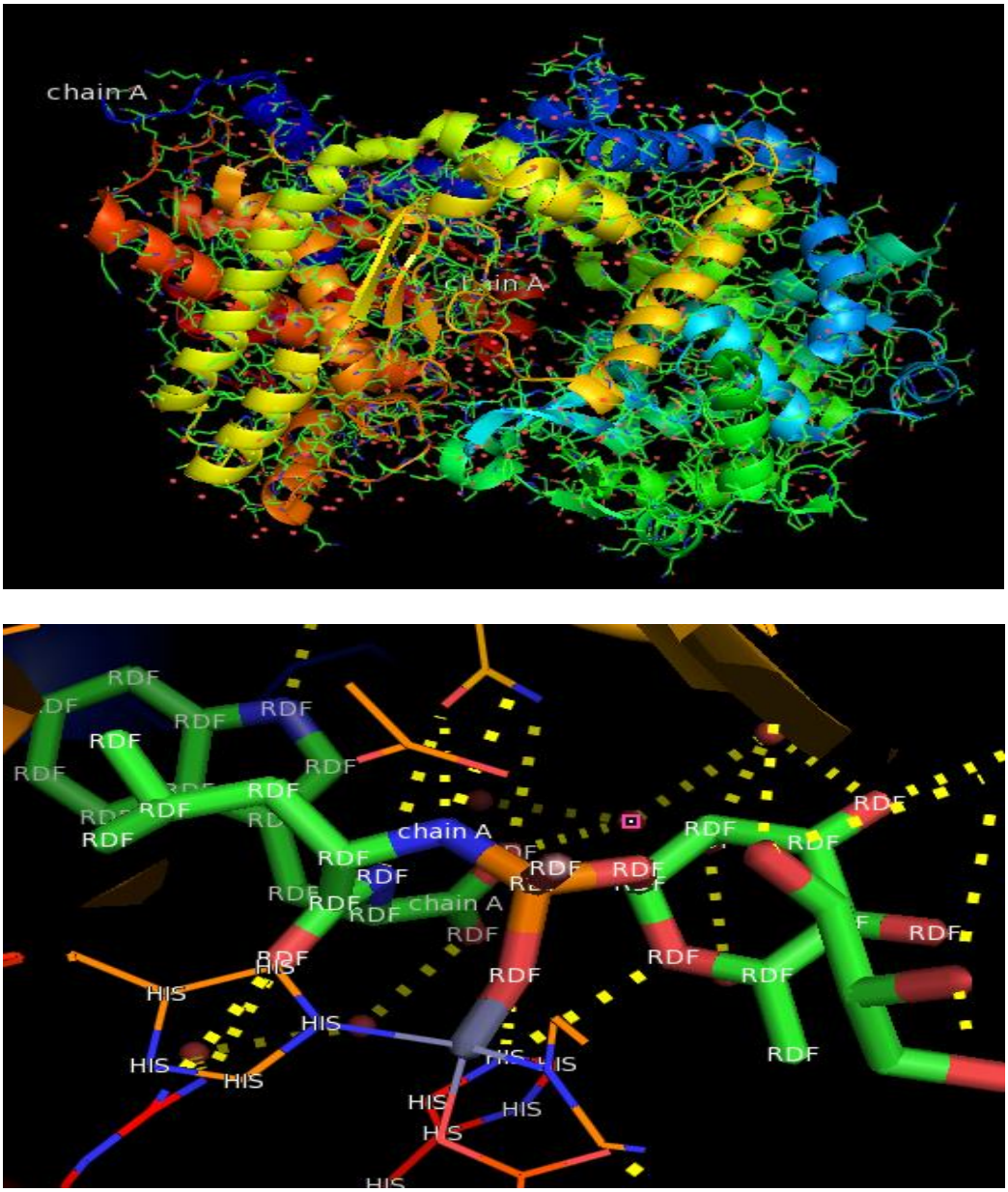


Figure 4: 3D Visualisation of Neprilysin (NEP) using Pymol

Protein-Protein Interaction:

For analyzing the protein-protein interaction, string online tool was used (https://string-db.org/). String provided us with various interacting partners of AβPP viz INS, MAPK8, CASP6, SNCA, BACE1 and PSEN1.

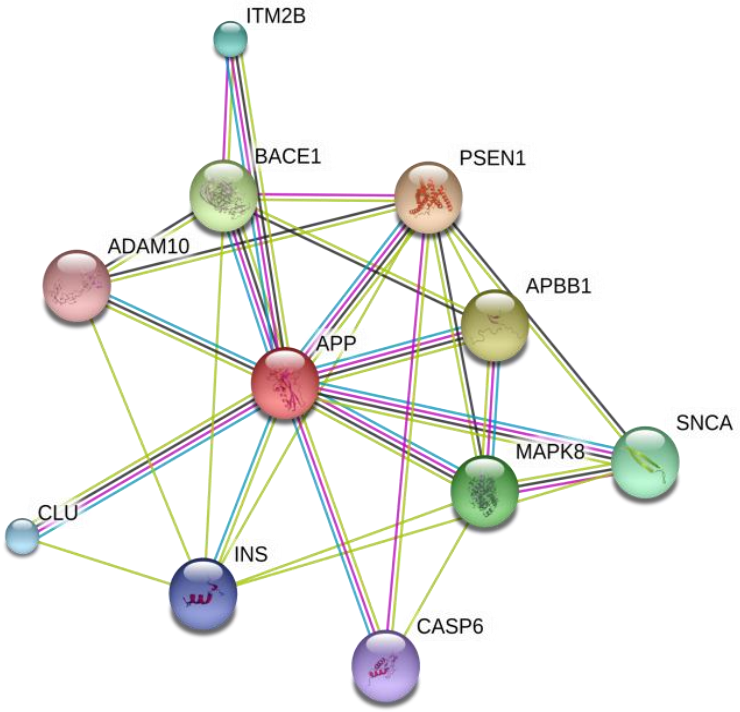
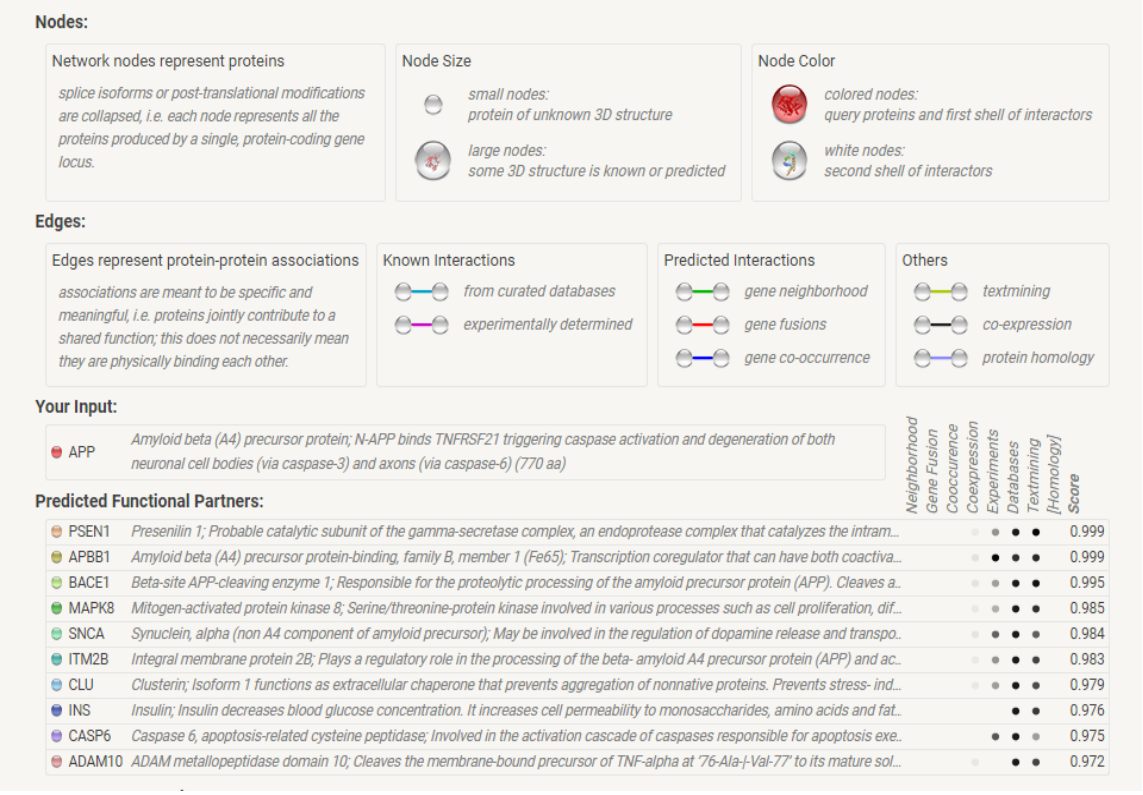


Figure 5: PPI network of AβPP viz INS, MAPK8, CASP6, SNCA, BACE1 and PSEN1



Screening of biomolecules for drug-likeliness and ADMET Analysis of compounds

The biomolecules are first tested for their drug-likeliness via Lipinski filti based on Lipinski rule of five. These all compounds that passed the Lipinski filter are further tested for their pharmacokinetic properties so as to get other parameters, namely Lipinski, Ghose and Veber, as well. The ADMET ananlysis revealed that all the compounds selected possessing drug likeliness can be used for docking purposes. Most importantly, they can cross the BBB and has high pharmacokinetics values **(Table 2)**.

Table 2: Differentiation of drugs on the basis of Lipinski Rule of five by Lipinski Filter

BIOMOLECULES	MASS	HYDROGEN BOND DONOR	HYDROGEN BOND ACCEPTOR	LOGP	MOLAR REFRACTIVITY	PASS/FAIL
CURCUMIN	372.000000	0	6	3.343619	95.534981	Pass
APOMORPHINE	263.000000	0	2	1.909650	73.646996	Pass
NOBILETIN	406.000000	0	8	3.644999	100.685989	Pass
RESVERATROL	228.000000	0	3	1.713190	58.199493	Pass
NOREPINEPHRINE	176.000000	2	3	2.104720	49.524895	Pass
IMATINIB	489.000000	0	2	2.330750	134.279312	Fail
EGCG	466.000000	0	11	3.312589	106.702484	Fail
PROPANOLOL	266.000000	0	2	3.654879	85.431984	Pass
SURAMIN	312.000000	5	6	0.053101	77.145782	Pass
TRICHOSTATIN A	310.000000	1	3	2.532070	94.904678	Pass

Table 3: ADMET analysis of selected compounds

Biomolecules	GI Permeability	Log S (ESOL)	BBB Permeability	Bioavailability score	Drug likeliness (Ghose,veber)
Curcumin	High	-3.94	No	0.55	Yes
Apomorphine	High	-3.39	Yes	0.55	Yes
Nobiletin	High	-4.18	No	0.55	Yes
Resveratrol	High	-3.62	Yes	0.55	Yes
Norepinephrine	High	-0.35	No	0.55	Yes

Active site prediction

Out of top nine pockets, IDE had best pocket at P2 with a drugability score of 0.87 and 0.02 standard deviation (Table 3). The volume of given pocket was 669.21 cubic angstroms and fifteen residues were involved in interaction at this site. Likewise, NEP had best pocket at P15 with a drugability score of 0.92 and 0.02 standard deviation (Table 4). The volume of given pocket was 1377.7 cubic angstroms and twenty residues were involved in interaction at this site.

Pockets	Vol. Hull*	Hydroph. Kyte*	Polar Res.*	Aromatic Res.*	Otyr atom	Nb. Res.*	Drugg Prob*	Standard Deviation
P 0	1264.72	-0.43	0.53	0.16	0.0	19.0	**0.62**	**0.04**
P 1	971.6	-0.39	0.42	0.05	0.0	19.0	**0.5**	**0.04**
P 2	669.21	0.39	0.4	0.07	0.0	15.0	**0.87**	**0.02**
P 3	1588.58	-0.86	0.6	0.1	0.0	20.0	0.32	0.01
P 4	392.46	-0.95	0.62	0.15	0.0	13.0	0.25	0.02
P 5°	*493.13*	*-0.97*	*0.6*	*0.0*	*0.0*	*10.0*	*0.15*	*0.05*
P 6°	*278.15*	*-0.09*	*0.5*	*0.0*	*0.0*	*8.0*	***0.56***	***0.09***
P 7°	*394.07*	*-1.54*	*0.7*	*0.1*	*0.0*	*10.0*	*0.06*	*0.01*
P 8°	*288.15*	*-1.42*	*0.5*	*0.0*	*0.0*	*10.0*	*0.05*	*0.02*

Table 4: Active sites of Insulin degrading enzyme

Pockets	Vol. Hull*	Hydroph. Kyte*	Polar Res.*	Aromatic Res.*	Otyr atom	Nb. Res.*	Drugg Prob*	Standard Deviation
P 0	6804.73	-0.73	0.58	0.25	0.01	52.0	**0.73**	**0.08**
P 1	1456.38	-1.31	0.59	0.09	0.0	22.0	0.12	0.01
P 10	1206.37	-1.64	0.76	0.06	0.0	17.0	0.05	0.01
P 11	953.17	-2.55	1.0	0.13	0.05	15.0	0.02	0.01
P 15	1377.7	0.1	0.65	0.2	0.04	20.0	**0.92**	**0.02**
P 16	793.29	-1.46	0.67	0.11	0.0	18.0	0.09	0.0
P 2	1427.87	-0.98	0.62	0.1	0.0	21.0	0.25	0.01
P 27	866.9	-1.56	0.71	0.14	0.03	14.0	0.12	0.05
P 3	978.68	-0.26	0.58	0.21	0.0	19.0	**0.74**	**0.07**

Table 5: Active sites of Neprilysin

Docking Calculations of biomolecules with both IDE and NEP:

Biomolecules bound to IDE at P2 pocket and same residues as predicted were involved in the interaction. The estimated free energy of binding for IDE and resveratrol was -5.38 kcal/mol and total intermolecular energy was -6.88 kcal/mol. Similarly, the estimated free energy of binding for IDE and curcumin was -2.43kcal/mol and total intermolecular energy was -4.75 kcal/mol. Likewise, the estimated free energy of binding for IDE and apomorphine was -4.61kcal/mol and total intermolecular energy was -4.6 kcal/mol. Further, estimated free energy of binding for IDE and nobiletin was -4.55 kcal/mol and total intermolecular energy was -6.39kcal/mol. Finally, estimated free energy of binding for IDE and norepinephrine was -3.63 kcal/mol and total intermolecular energy was -4.14 kcal/mol. Molecular docking pattern of IDE with screened have been identified and depicted in **Figure 6**. On the basis of docking analysis, interacting compounds with minimum binding constant and highest negative free energy of binding are most effective. Docking calculation of IDE with these molecules has been presented in **Table 6.** Additionally, HB *plot* was generated for studying hydrogen bond networks involved between

IDE and selected biomolecules during docking study **(Figure 7)**. Further, this analysis of protein structures yields significant new information on protein function.

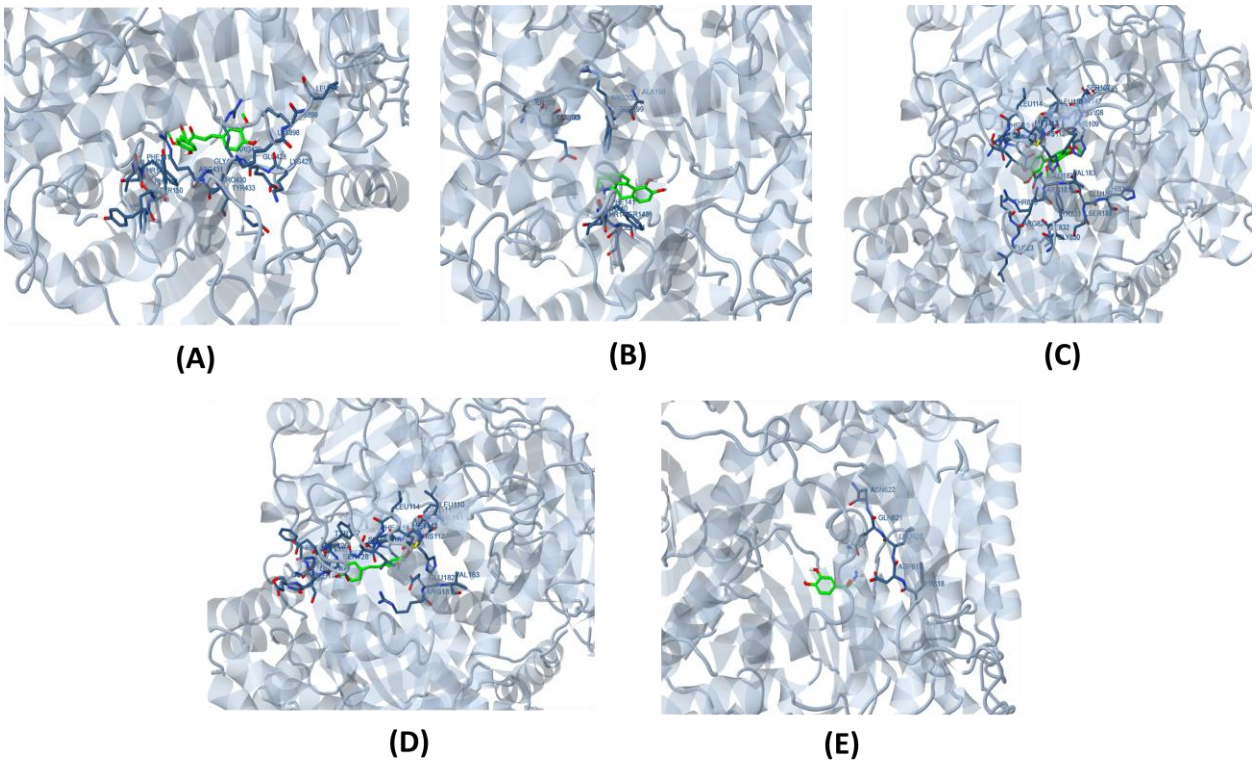


Figure 6: Binding of Insulin degrading enzyme with selected compounds (A) Curcumin, (B) Apomorphine, (C) Nobiletin, (D) Resveratrol, and (E) Norepinephrine

S. No.	Biomolecules	Est. free energy of binding (kcal/mol)	Est. inhibition constant, Ki (uM)	VdW+Hbond+dissolve energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total intermolecular energy (kcal/mol)	Frequency (in %)	Interact. Surface
1	Curcumin	-2.43	16.63	-4.47	-0.28	-4.75	10	587.823
2	Apomorphine	-4.61	418.97	-3.9	-0.7	-4.6	50	487.742
3	Nobiletin	-4.55	460.99	-6.05	-0.34	-6.39	30	792.235
4	Resveratrol	-5.38	112.92	-6.8	-0.08	-6.88	40	660.852
5	Norepinephrine	-3.63	2.20	-1.95	-2.19	-4.14	30	161.03

Table 6: Docking calculation of biomolecules with IDE

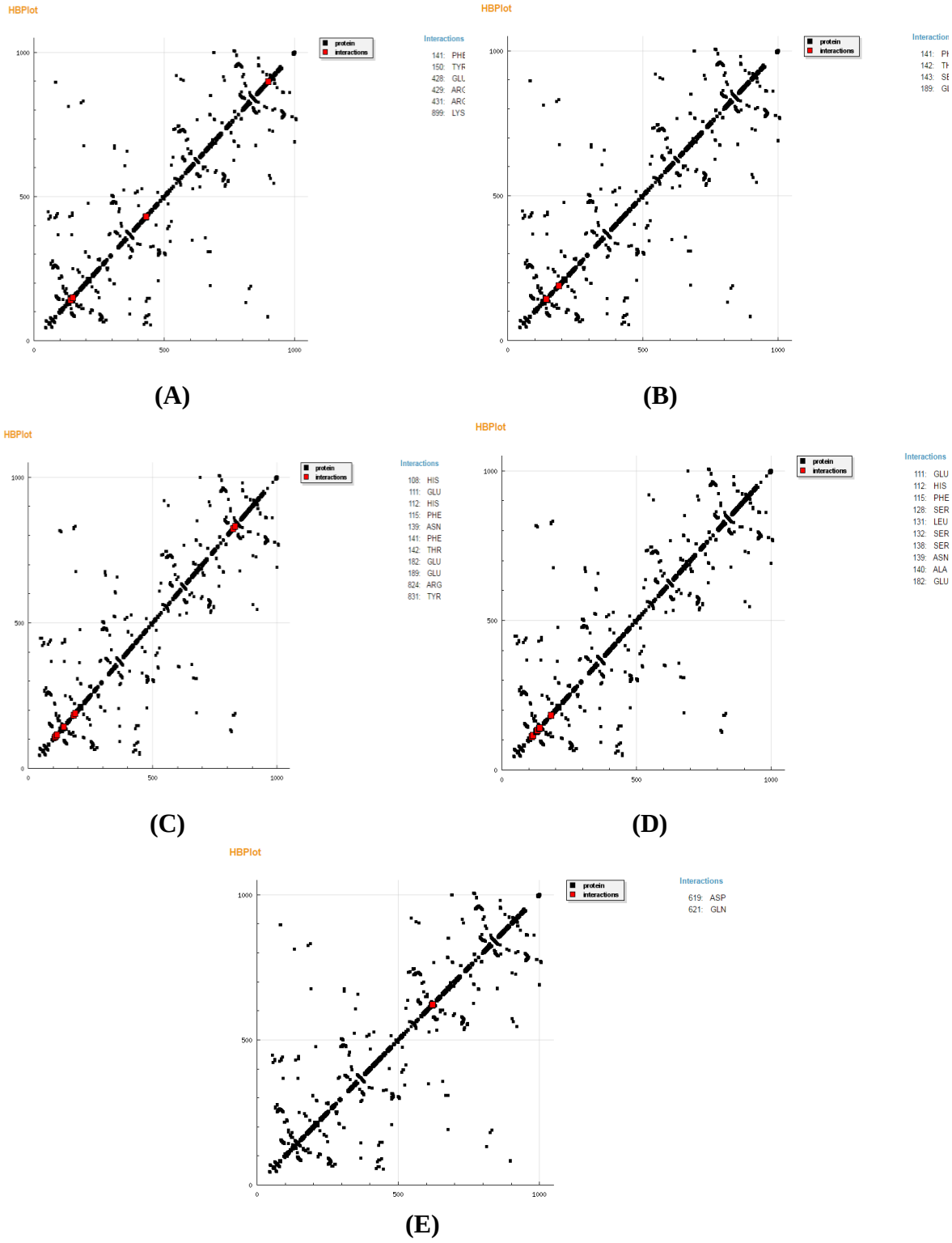


Figure 7: HB plot results of Insulin degrading enzyme with selected compounds (A) Curcumin, (B) Apomorphine, (C) Nobiletin, (D) Resveratrol, and (E) Norepinephrine

Further, biomolecules bound to NEP at P15 pocket and same residues as predicted were involved in the interaction. The estimated free energy of binding for NEP and curcumin was -3.96 kcal/mol and total intermolecular energy was -6.16 kcal/mol. Likewise, The estimated free energy of binding for NEP and apomorphine was -7.19 kcal/mol and total intermolecular energy was -7.16 kcal/mol. Further, estimated free energy of binding for NEP and nobiletin was -4.81 kcal/mol and total intermolecular energy was -6.73 kcal/mol. While, estimated free energy of binding for NEP and resveratrol was -4.05 kcal/mol and total intermolecular energy was -5.54 kcal/mol. Finally, estimated free energy of binding for NEP and norepinephrine was -6.28 kcal/mol and total intermolecular energy was -6.98 kcal/mol. Molecular docking pattern of NEP with screened have been identified and depicted in **Figure 8**. On the basis of docking analysis, interacting compounds with minimum binding constant and highest negative free energy of binding are most effective. Docking calculation of NEP with these molecules has been presented in **Table 7.** Additionally, HB *plot* was generated for studying hydrogen bond networks involved between NEP and selected biomolecules during docking study **(Figure 9)**. Further, this analysis of protein structures yields significant new information on protein function.

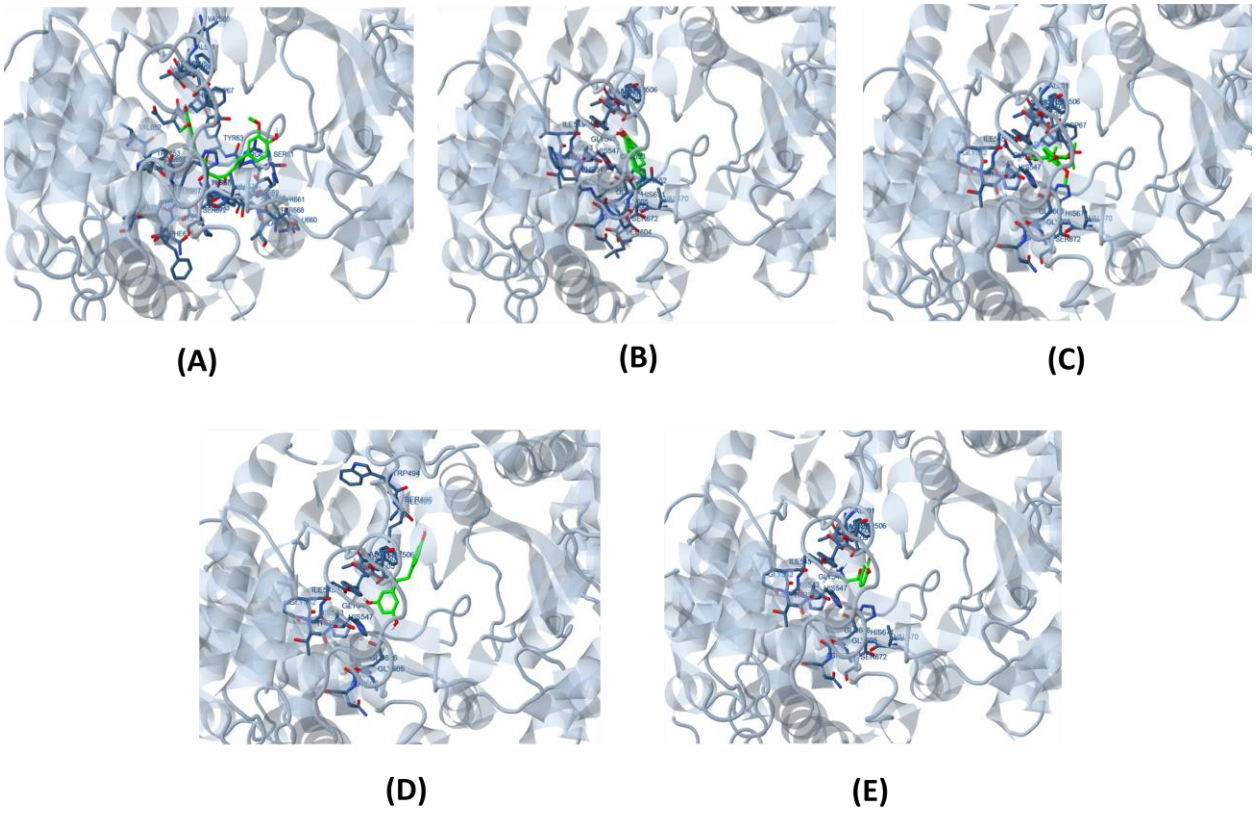


Figure 8: Binding of NEP with selected compounds (A) Curcumin, (B) Apomorphine, (C) Nobiletin, (D) Resveratrol, (E) Norepinephrine

S. No.	Biomolecules	Est. free energy of binding (kcal/mol)	Est. inhibition constant, Ki (uM)	VdW+Hbond+dissolve energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total intermolecular energy (kcal/mol)	Frequency (in %)	Interact. surface
1	Curcumin	-3.96	1.26	-6.28	+0.12	-6.16	10	909.403
2	Apomorphine	-7.19	5.35	-5.57	-1.59	-7.16	20	592.664
3	Nobiletin	-4.81	300.09	-6.34	-0.39	-6.73	10	850.792
4	Resveratrol	-4.05	1.07	-5.01	-0.53	-5.54	20	479.247
5	Norepinephrine	-6.28	25.10	-4.32	-2.66	-6.98	30	418.641

Table 7: Docking calculation of biomolecules with NEP

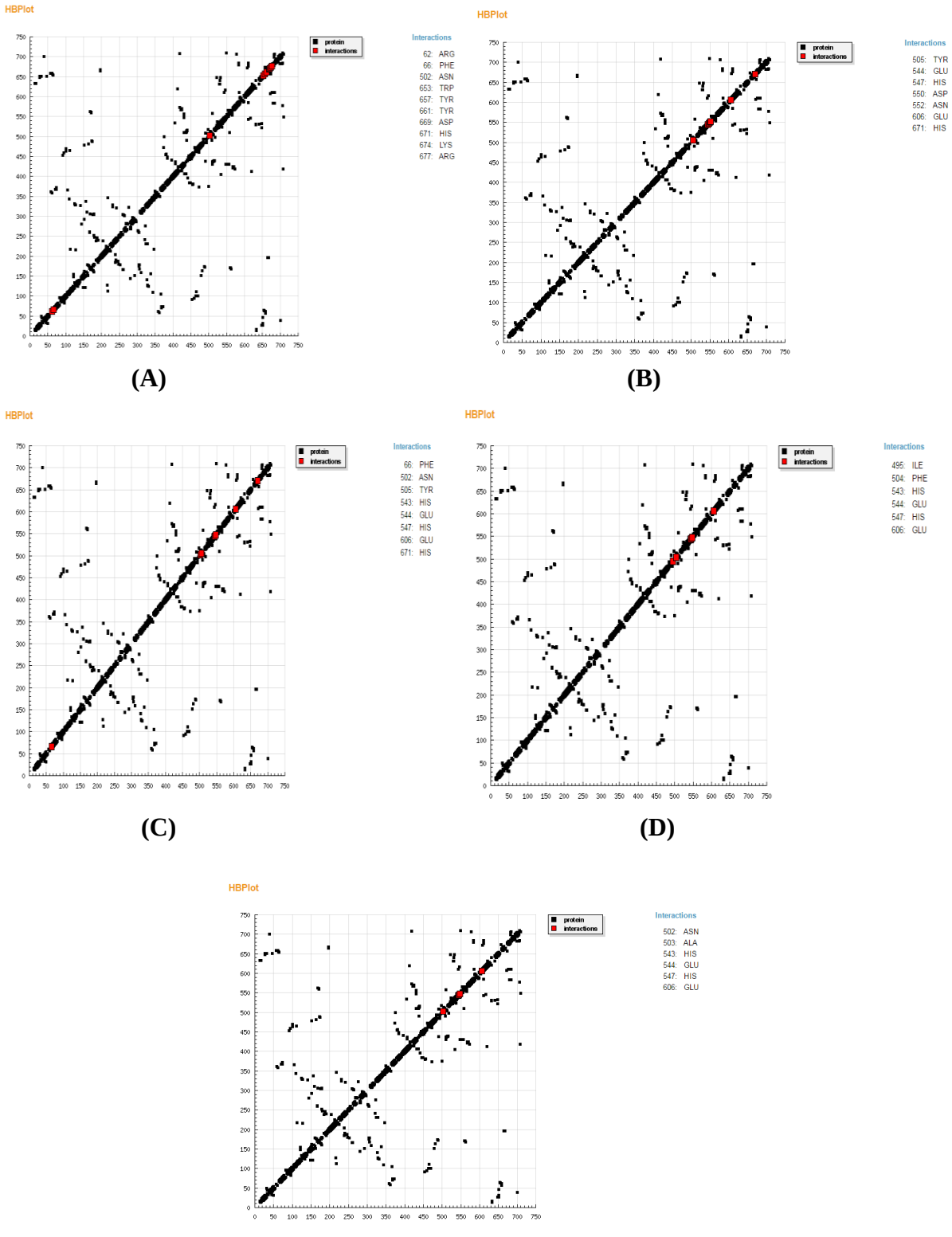


Figure 9: HB plot results of Neprilysin with selected compounds (A) Curcumin, (B) Apomorphine, (C) Nobiletin, (D) Resveratrol, (E) Norepinephrine

DISCUSSION

The biology behind Aβ deposition in the brain and the clearance mechanism is quite complicated, and thus much attention has been given in the past few decades to understand these phenomena and their significance in AD [64]. The etiopathology of AD is caused by accumulation of toxic Aβ peptides that occurred due to several factors, mainly associated with Proteolytic cleavage of AβPP by γ-secretase, that lead to the formation of AICD and toxic Aβ [65]. Protective enzymes such as IDE and NEP are found to play a crucial role in degradation of amyloidogenic or toxic Aβ peptides in the brain, thus signifying their therapeutic potential [66]. Conversely, higher expression of both IDE and NEP reportedly cause numerous cancers including Breast cancer. Therefore, it seems imperative to design therapeutic strategies aimed at controlling the expression of both IDE and NEP to inhibit the cascade of neuronal death and cancer.

Various biomolecules such as apomorphine, a kind of dopamine receptor agonists accountable for promoting the intracellular Aβ degradation via activating IDE [67]. Curcumin increases Aβ clearance by increasing both IDE and NEP activity and also prevents Aβ production by inhibiting PS2, a catalytic component of γ-secretase. Similarly, Nobiletin enhanced NEP expression both at the gene and protein level in time- and dose-dependent manner in SK-N-SH cells and thereby promoting Aβ clearance. Further, Resveratrol significantly increases both the estradiol and NEP level that decrease Aβ deposition; by upregulation of estradiol level which consequently leads to increase the level of NEP, thus contribute to Aβ degradation [1]. Taken together, all these data provide convincing evidence of using these biomolecules in modulating the level of of both IDE and NEP and in turn, inhibit the cascade of neuronal death.

In the present study, we tested the drug-able efficacy of curcumin, apomorphine, nobiletin, resveratrol, and norepinephrine in regulating both IDE and NEP expression in the brain. Since normal expression of these proteins protect neuronal cells from synaptic death while its higher expression causes numerous cancer including breast cancer. Emphasis was laid on pharmacokinetic analysis as aqueous solubility and dissolution in GI fluids are defining parameters of *in vivo* bioavailability of an orally administered drug [68]. Similarly, lipophilicity of a drug directs physiological properties such as rate of metabolism, transport across cell membrane and interaction with binding sites of receptor. Further, CNS drugs should have logP value less than 4 [68-70]. The logP value for these selected biomolecules was found to be less than 4.

However, the most important property required of a compound or biomolecules intended to be a neuroprotective agent is BBB permeability. The selected biomolecules for our study qualified all the above mentioned parameters and scored well on pharmacokinetics, bioavailability score and could cross the BBB. Finally, molecular docking studies indicated that the selected biomolecules for our study can bind to and control the level of both IDE and NEP and possibly, halt or inhibit both neurodegeneration and oncogenic advancement. These findings can be validated through *in vitro* and *in vivo* studies in near future.

CONCLUSION

The results of our study provide novel potential of biomolecules such as curcumin, apomorphine, nobiletin, resveratrol, and norepinephrine in regulating both IDE and NEP expression in the brain, which has wider implications in the progression as well as protection against NDDs. Out of these five biomolecules resveratrol is showing better interaction with IDE based on their

minimum binding constant and highest negative free energy. Whereas, norepinephrine is showing better interaction with NEP based on their minimum binding constant and highest negative free energy. Further, both *in vivo* and *in vitro* study is needed to validate these results in more convincing way.

REFERENCES

[1] Jha NK, Jha SK, Kumar D, Kejriwal N, Sharma R, Ambasta RK, Kumar P. Impact of Insulin Degrading Enzyme and Neprilysin in Alzheimer's Disease Biology: Characterization of Putative Cognates for Therapeutic Applications. *J Alzheimers Dis*. 2015;48(4):891-917

[2] Zhang S, Chai R, Yang YY, Guo SQ, Wang S, Guo T, Xu SF, Zhang YH, Wang ZY, Guo C. Chronic diabetic states worsen Alzheimer neuropathology and cognitive deficits accompanying disruption of calcium signaling in leptin-deficient APP/PS1 mice. *Oncotarget*. 2017. doi: 10.18632/oncotarget.17116

[3] Andrés RC, Helena BC, Juliana PP, Viviana AM, Margarita GB, Marisa CG. Diabetes-related neurological implication and pharmacogenomics. *Curr Pharm Des*. 2017. doi: 10.2174/1381612823666170317165350

[4] Nalivaeva NN, Turner AJ. Role of Ageing and Oxidative Stress in Regulation of Amyloid-Degrading Enzymes and Development of Neurodegeneration. *Curr Aging Sci*. 2017;10(1):32-40

[5] Wang S, Wang R, Chen L, Bennett DA, Dickson DW, Wang DS. Expression and functional profiling of neprilysin, insulin-degrading enzyme, and endothelin-converting enzyme in prospectively studied elderly and Alzheimer's brain. *J Neurochem*. 2010;115(1):47-57

[6] Kurochkin IV, Guarnera E, Wong JH, Eisenhaber F, Berezovsky IN. Toward Allosterically Increased Catalytic Activity of Insulin-Degrading Enzyme against Amyloid Peptides. *Biochemistry*. 2017;56(1):228-239

[7] Jakki SL, Senthil V, Yasam VR, Chandrasekar M, Vijayaraghavan C. The blood brain barrier and its role in Alzheimer's therapy: An overview. *Curr Drug Targets*. 2017 Jun 12

[8] Zoltowska KM, Berezovska O. Dynamic Nature of presenilin1/γ-Secretase: Implication for Alzheimer's Disease Pathogenesis. *Mol Neurobiol*. 2017 Mar 22. doi: 10.1007/s12035-017-0487-5

[9] cAMP-responsive element-binding protein mediated transcription, agedependent axon pathology, and premature death in Drosophila. *J Biol Chem* 2008;283:19066Y76

[10] Dona O, Thompson J, Druchok C. Comprehensive Review on Magnetic Resonance Imaging in Alzheimer's Disease. *Crit Rev Biomed Eng*. 2016;44(3):213-225

[11] Jakki SL, Senthil V, Yasam VR, Chandrasekar M, Vijayaraghavan C. The blood brain barrier and its role in Alzheimer's therapy: An overview. *Curr Drug Targets*. 2017

[12] Donohue MC, Sperling RA, Petersen R, Sun CK, Weiner MW, Aisen PS; Alzheimer's Disease Neuroimaging Initiative. Association Between Elevated Brain Amyloid and Subsequent Cognitive Decline Among Cognitively Normal Persons. *JAMA.* 2017 Jun 13;317(22):2305-2316

[13] Sun CF, Xu LT, Li ZJ. [Diagnosis, surgical treatment and prognosis of primary tracheobronchial adenoid cystic carcinoma]. *Zhonghua Wai Ke Za Zhi*. 1987 Oct;25(10):586-8, 613

[14] Zoltowska KM, Berezovska O. Dynamic Nature of presenilin1/γ-Secretase: Implication for Alzheimer's Disease Pathogenesis. *Mol Neurobiol*. 2017 Mar 22. doi: 10.1007/s12035-017-0487-5

[15] Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. *Science* 1992;256:184Y85

[16] Selkoe DJ. The molecular pathology of Alzheimer's disease. *Neuron* 1991;6:487Y98

[17] Weller RO, Subash M, Preston SD, et al. Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. *Brain Pathol* 2008;18:253Y66

[18] Deane R, Sagare A, Zlokovic BV. The role of the cell surface LRP and soluble LRP in blood-brain barrier Abeta clearance in Alzheimer's disease. *Curr Pharm Des* 2008;14:1601Y5

[19] Carson JA, Turner AJ. Beta-amyloid catabolism: Roles for neprilysin (NEP) and other metallopeptidases? *J Neurochem* 2002;81:1Y8

[20] Turner AJ, Fisk L, Nalivaeva NN. Targeting amyloid-degrading enzymes as therapeutic strategies in neurodegeneration. *Ann N Y Acad Sci* 2004;1035:1Y20

[21] Hardy J. Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci* 1997;20:154Y59

[22] Tyler SJ, Dawbarn D, Wilcock GK, et al. alpha- and beta-secretase: Profound changes in Alzheimer's disease. *Biochem Biophys Res Commun* 2002;299:373Y76

[23] Zhao J, Fu Y, Yasvoina M, et al. Beta-site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: Implications for Alzheimer's disease pathogenesis. *J Neurosci* 2007;27:3639Y49

[24] Wang DS, Dickson DW, Malter JS. Beta-amyloid degradation and Alzheimer's disease. *J Biomed Biotechnol* 2006;2006:58406

[25] Eckman EA, Adams SK, Troendle FJ, et al. Regulation of steady-state beta-amyloid levels in the brain by neprilysin and endothelin-converting enzyme but not angiotensin-converting enzyme. *J Biol Chem* 2006;281:30471Y78

[26] Iwata N, Mizukami H, Shirotani K, et al. Presynaptic localization of neprilysin contributes to efficient clearance of amyloid-beta peptide in mouse brain. *J Neurosci* 2004;24:991Y98

[27] Iwata N, Tsubuki S, Takaki Y, et al. Metabolic regulation of brain Abeta by neprilysin. *Science* 2001;292:1550Y52

[28] Farris W, Mansourian S, Chang Y, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci USA* 2003;100:4162Y67

[29] Miller BC, Eckman EA, Sambamurti K, et al. Amyloid-beta peptide levels in brain are inversely correlated with insulysin activity levels in vivo. *Proc Natl Acad Sci USA* 2003;100:6221Y26

[30] Leissring MA, Farris W, Chang AY, et al. Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron* 2003;40:1087Y93

[31] Marr RA, Rockenstein E, Mukherjee A, et al. Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. *J Neurosci* 2003; 23:1992Y96

[32] Hemming ML, Patterson M, Reske-Nielsen C, et al. Reducing amyloid plaque burden via ex vivo gene delivery of an Abeta-degrading protease: A novel therapeutic approach to Alzheimer disease. *PLoS Med* 2007;4:e362

[33] El-Amouri SS, Zhu H, Yu J, et al. Neprilysin protects neurons against Abeta peptide toxicity. *Brain Res* 2007;1152:191Y200

[34] Poirier R, Wolfer DP, Welzl H, et al. Neuronal neprilysin overexpression is associated with attenuation of A(-related spatial memory deficit. Neurobiol Dis 2006;24:475Y83

[35] Carpentier M, Robitaille Y, DesGroseillers L, et al. Declining expression of neprilysin in Alzheimer disease vasculature: Possible involvement in cerebral amyloid angiopathy. *J Neuropathol Exp Neurol* 2002;61:849Y56

[36] Miners JS, Van Helmond Z, Chalmers K, et al. Decreased expression and activity of neprilysin in Alzheimer disease are associated with cerebral amyloid angiopathy. *J Neuropathol Exp Neurol* 2006;65:1012Y21

[37] Miners JS, Baig S, Palmer J, et al. Abeta-degrading enzymes in Alzheimer's disease. *Brain Pathol* 2008;18:240Y52

[38] Perez A, Morelli L, Cresto JC, et al. Degradation of soluble amyloid beta-peptides 1-40, 1-42, and the Dutch variant 1-40Q by insulin degrading enzyme from Alzheimer disease and control brains. *Neurochem Res* 2000;25:247Y55

[39] Zhao Z, Xiang Z, Haroutunian V, et al. Insulin degrading enzyme activity selectively decreases in the hippocampal formation of cases at high risk to develop Alzheimer's disease. *Neurobiol Aging* 2007;28:824Y30

[40] Hellstrom-Lindahl E, Ravid R, Nordberg A. Age-dependent decline of neprilysin in Alzheimer's disease and normal brain: Inverse correlation with Abeta levels. *Neurobiol Aging* 2008;29:210Y21

[41] Caccamo A, Oddo S, Sugarman MC, et al. Age- and region-dependent alterations in Abeta-degrading enzymes: Implications for Abeta-induced disorders. *Neurobiol Aging* 2005;26:645Y54

[42] Carter TL, Pedrini S, Ghiso J, et al. Brain neprilysin activity and susceptibility to transgene-induced Alzheimer amyloidosis. *Neurosci Lett* 2006;392:235Y39

[43] Cook DG, Leverenz JB, McMillan PJ, et al. Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. *Am J Pathol* 2003; 162:313Y19

[44] Harada H, Tamaoka A, Ishii K, et al. Beta-site APP cleaving enzyme 1 (BACE1) is increased in remaining neurons in Alzheimer's disease brains. Neurosci Res 2006;54:24Y29

[45] Miners JS, Verbeek MM, Rikkert MO, et al. Immunocapture-based fluorometric assay for the measurement of neprilysin-specific enzyme activity in brain tissue homogenates and cerebrospinal fluid. *J Neurosci Methods* 2008;167:229Y36

[46] Miners JS, Kehoe PG, Love S. Immunocapture-based fluorometric assay for the measurement of insulin-degrading enzyme activity in brain tissue homogenates. *J Neurosci Methods* 2008;169:177Y81

[47] Akiyama H, Kondo H, Ikeda K, et al. Immunohistochemical localization of neprilysin in the human cerebral cortex: Inverse association with vulnerability to amyloid beta-protein (Abeta) deposition. *Brain Res* 2001;902:277Y81

[48] Yasojima K, Akiyama H, McGeer EG, et al. Reduced neprilysin in high plaque areas of Alzheimer brain: A possible relationship to deficient degradation of beta-amyloid peptide. *Neurosci Lett* 2001;297:97Y100

[49] Wang DS, Lipton RB, Katz MJ, et al. Decreased neprilysin immunoreactivity in Alzheimer disease, but not in pathological aging. *J Neuropathol Exp Neurol* 2005;64:378Y85

[50] Russo R, Borghi R, Markesbery W, et al. Neprylisin decreases uniformly in Alzheimer's disease and in normal aging. *FEBS Lett* 2005;579:6027Y30

[51] Maruyama M, Higuchi M, Takaki Y, et al. Cerebrospinal fluid neprilysin is reduced in prodromal Alzheimer's disease. *Ann Neurol* 2005;57:832Y42

[52] Apelt J, Ach K, Schliebs R. Aging-related down-regulation of neprilysin, a putative beta-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial Upregulation in the vicinity of beta-amyloid plaques. *Neurosci Lett* 2003;339:183Y86

[53] Leal MC, Dorfman VB, Gamba AF, et al. Plaque-associated overexpression of insulin-degrading enzyme in the cerebral cortex of aged transgenic tg2576 mice with Alzheimer pathology. *J Neuropathol Exp Neurol* 2006;65:976Y87

[54] Vepsalainen S, Hiltunen M, Helisalmi S, et al. Increased expression of Abeta degrading enzyme IDE in the cortex of transgenic mice with Alzheimer's disease-like neuropathology. *Neurosci Lett* 2008;438:216Y20

[55] Deb S, Zhang JW, Gottschall PE. Activated isoforms of MMP-2 are induced in U87 human glioma cells in response to beta-amyloid peptide. *J Neurosci Res* 1999;55:44Y53

[56] Jung SS, Zhang W, Van Nostrand WE. Pathogenic A beta induces the expression and activation of matrix metalloproteinase-2 in human cerebrovascular smooth muscle cells. *J Neurochem* 2003;85:1208Y15

[57] Lee JM, Yin KJ, Hsin I, et al. Matrix metalloproteinase-9 and spontaneous hemorrhage in an animal model of cerebral amyloid angiopathy. *Ann Neurol* 2003;54:379Y82

[58] Tucker HM, Kihiko-Ehmann M, Wright S, et al. Tissue plasminogen activator requires plasminogen to modulate amyloid-beta neurotoxicity and deposition. *J Neurochem* 2000;75:2172Y77

[59] Pardossi-Piquard R, Petit A, Kawarai T, et al. Presenilin-dependent transcriptional control of the Abeta-degrading enzyme neprilysin by intracellular domains of betaAPP and APLP. *Neuron* 2005;46:541Y54

[60] Chen AC, Selkoe DJ. Response to: Pardossi-Piquard et al. B Presenilin Dependent Transcriptional Control of the Abeta-Degrading Enzyme Neprilysin by Intracellular Domains of betaAPP and APLP.[Neuron 46, 541Y554. *Neuron* 2007;53:479Y83

[61] Ganju RK, Shpektor RG, Brenner DG, et al. CD10/neutral Endopeptidase 24.11 is phosphorylated by casein kinase II and coassociates with other phosphoproteins including the lyn srcYrelated kinase. *Blood* 1996; 88:4159Y65

[62] Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. *J Neurochem* 2008;104:1433Y39 51.

[63] Grouselle D, Winsky-Sommerer R, David JP, et al. Loss of somatostatin like immunoreactivity in the frontal cortex of Alzheimer patients carrying the apolipoprotein epsilon 4 allele. *Neurosci Lett* 1998;255:21Y24

[64] Wyss-Coray T, Rogers J. Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. *Cold Spring Harb Perspect Med*. 2012;2(1):a006346

[65] O'Brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. *Annu Rev Neurosci*. 2011;34:185-204

[66] Wang DS, Dickson DW, Malter JS. beta-Amyloid degradation and Alzheimer's disease. *J Biomed Biotechnol.* 2006;2006(3):58406

[67] Steele JW, Gandy S. Apomorphine and Alzheimer Aβ: roles for regulated α cleavage, autophagy, and antioxidation? Ann Neurol. 2011;69(2):221-5

[68] Khadka P, Jieun R, Kim H, Kim I, Kim JT, Kim H, et al. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian Journal of Pharmaceutical Sciences 2014;9(6):304–16

[69] Chico LK, Van Eldik LJ, Watterson DM. Targeting protein kinases in central nervous system disorders. Nat Rev Drug Discov 2009;8(11):892-09

[70] Wager TT, Hou X, Verhoest PR, Villalobos A. Moving beyond rules: the development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. ACS Chem Neurosci 2010;1(6):435-49