Application of Biomolecules in Huntington's disease: an *in silico* analysis of huntingtin (htt) gene with drugs

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Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled "**Application of Biomolecules in Huntington's disease: an *in silico* analysis of huntingtin (htt) gene with drugs"** submitted by **Shailesh Kumar Singh (DTU/14/M.TECH/109)** in the partial fulfilment 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 him 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, **Shailesh Kumar Singh** hereby declare that the dissertation entitled "**Application of Biomolecules in Huntington's disease: an *in silico* analysis of huntingtin (htt) gene with drugs"** 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 "Molecular Neuroscience and Functional Genomics Laboratory", 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: **Shailesh Kumar Singh**

Place: New Delhi DTU/14/M.TECH/109

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I Shailesh Kumar Singh, student of M.Tech- Biomedical Engineering, registration number-DTU/14/M.TECH/109 is presenting a project report on **"Application of Biomolecules in Huntington's disease: an *in silico* analysis of huntingtin (htt) gene with drugs"**. I would like to express my special thanks of gratitude to my mentor, **Dr. Pravir Kumar** for providing this wonderful opportunity to do this project. I also like to thank **Prof. D. Kumar** (Head of Department) and all faculty members of Department of Biotechnology, Delhi Technological University for their cooperation.

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LIST OF ABBREVIATIONS

CYP	Cytochrome P450
TBZ	Tetrabenazine
GABA	Gamma-amino butyric acid
NMDA-R	N-methyl D-aspartate Receptor
MMPs	Matrix metalloproteinases
FDA	Food and Drug Administration
PDB	Protein Data Bank

Application of Biomolecules in Huntington's disease: an *in silico* analysis of huntingtin (htt) gene with drugs

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1. ABSTRACT

Huntington's disease (HD) is an inherited progressive and severe neurodegenerative disorder that commonly starts in mid age, predominantly in striatum and is described by involuntary movements, personality changes and dementia. The mutated gene responsible for expression of polyglutamine repeats in huntingtin proteins, Contains a trinucleotide CAG repeat expansion within its coding region. The molecular mechanisms involved in cell death due to the toxic effect of mutant huntingtin is unknown, but a strong body of evidence shows that mutant protein in HD misfolds and accumulate into aggregate and the huntingtin protein is fragmented in affected individuals, htt exon1, a fragment of huntingtin which forms amyloid fibril aggregates, that might be cause of toxicity. Tetrabenazine (TBZ) is the only US Food and Drug approved drug for HD patient. The objective of this study is to identify newer antipsychotic biomolecules with adequate efficacy and more favorable adverse effect than tetrabenazine. Some natural products like Curcumin, Berberine, Aripiprazole, Clozapine, Olanzapine, Pridopidine, Quetiapine, Rilmenidine and Tiapride are shown to be effective in relieving the adverse effect of accumulated misfolded proteins in several neurodegenerative diseases. Hence, these natural products can be promising therapeutic approach to Huntington's disease (HD). Here, *in silco* molecular docking was performed against huntingtin gene (htt) using antipsychotic biomolecules and furthermore docking results was compared with docking result of tetrabenazine.

2. INTRODUCTION

Huntington's disease (HD) is an autosomal dominant disorder of the nervous System caused by mutation in the htt gene located at chromosome no 4. It is characterized by progressive motor, cognitive, dementia and behavioral impairment. The defective gene produces mutant huntingtin protein containing repeat of glutamine amino acid expansion in the N-terminal portion. In the brain, htt is mainly expressed in neurons, predominantly in striatum (Sapp *et al.*, 1997). The main site of deterioration in person with HD is the striatum; although other parts of brain are also affected at later stages (Vonsattel *et al.,* 1995).symptoms of HD commonly appear in middle stage of life. However, disease can start in early stages, and around 6% of HD patients acquire this disease in early ages (Foroud *et al.,* 1999).The primary symptoms vary from person to person but disease onset is generally noticed by uncontrolled movements of the finger, face, thorax or feet (Folstein *et al.,* 1996). As HD develops, the affected person develops overt choreiform movements of the head, neck, arms and legs. Individual with HD also show cognitive decline such as impairments of language comprehension and memory and its severity depends upon disease progression (Craufurd, D. 2001). Weight loss is a 4^{th} symptom of the disease and may be due to dysphagia as well as degeneration of hypothalamic orexin-positive neurons (Bachoud *et al.,* 2001; Petersen *et al.,* 2005). After few years of the disease progression, individual becomes completely rigid and akinetic. They also present severe dementia, eventually become unable to talk and can't care for themselves. The patient usually dies 10–20 years after the first symptoms appear as there is currently no treatment available to check or delay disease progression. The neuropathology of HD involves the selectively loss of function and death of specific neuron within the central nervous system. The most affected cells are neurons of the striatum that releases gamma-amino butyric acid (GABA), the subcortical brain structure that controls different cognitive process and, to a lesser extent, neurons within the cerebral cortex (Li *et al.,* 2003). The first degenerating subpopulation of GABAergic neurons express encephalin and are enriched in the dopamine receptor D2 (Vonsattel *et al.,* 1985; Graveland *et al.,* 1985). As the disease grows older, there is general neuronal loss in different brain region like the *globus pallidus*, the *substantia nigra*, the subthalamic nuclei, the cerebellum and the thalamus. Glial proliferation is also observed Together with the neuronal loss (Li *et al.,* 2003; Sapp *et al.,* 1995), but the region is still not clear.

3. LITERATURE REVIEW

3.1 Huntington's Disease

Huntington's disease (HD) is hereditary neurological disorder characterized by behavioral impairment, abnormal involuntary movement, psychiatric disorders and intellectual deterioration (Martin *et al.,* 1986). The post-mortem examination of tissues from HD patients revealed that striatum is predominantly affected, although neuropathological changes have also been detected in other areas of the brain such as the cerebellar cortex, thalamus and cerebellum (Reiner *et al.,* 1988; Rosas *et al.,* 2003). HD is caused by abnormal CAG expansion in the huntingtin gene (htt) located on chromosome 4. In healthy humans, typically 6 to 35 CAG repeats are found at the exon-1 of htt gene, whereas in HD patients CAG repeats are described more than 40 trinucleotides. In most of the cases, an intermediate CAG repeats (36–40) causes a slower progression of the pathology as a result of the inadequate penetrance of the mutant allele. Essentially, the onset and severity of the pathology directly depends upon the number of CAG repeats in htt gene, although the real function of the trinucleotide stretch is not clear yet (Andrew *et al.,* 1993; Rubinsztein *et al.,* 1993). Recent findings reported that size of the CAG repeats stretch might be important in the translation of the mRNA transcript of htt, due to result of association with a ribosome-containing complex7. The htt gene encodes a protein of an approximately 350 kDa, containing several subdomains. The polyglutamine (polyQ) stretch present at the N-terminus of htt gene, encoded by the CAG repeats works as potential membrane association signal (Atwal *et al.,* 2007). In mammals, polyproline sequence followed the polyQ containing domain that stabilizes protein functional conformation. The N-terminal section of htt gene is followed by three clusters of HEAT repeats; HEAT repeats are very vital for the binding with interacting proteins. Along with these important motifs, htt gene contains many different sites for posttranslational modifications. Gene encoding htt protein has been identified in the nucleus, mitochondria, Golgi and endoplasmic reticulum of the cell and can be found in the neuronal body, dendrites and synapses (DiFiglia *et al.,* 1995; Trottier *et al.,* 1995). There is evidence that htt gene interacts with a range of proteins at molecular level, such as cytoskeleton proteins and transcriptional factors (Zuccato *et al.,* 2010). htt gene is ubiquitously expressed at the time of embryonic growth and at very high levels in testis and in mature postmitotic neurons in adult human brain (Strong *et al.,* 1993).

3.2 Epidemiology

Huntington's disease is mostly seen in European populations at prevalence rate of 4–8 cases per 100,000, and it is commonly found in India and other parts of Central Asia (Harper, PS. 1992). This prevalence rate has confirmed by current studies in other European nations (Morrisone *et al.,* 1995; Peterlin *et al.,* 2009). HD is rarely found in some countries like Finland and Japan, but adequate data is not available for Eastern Asia and African countries. A recent study revealed, Mexico has slightly higher percentage of juvenile cases and higher prevalence rate of other form of HD than expected (Alonso *et al.,* 2009). Scotland and the Lake Maracaibo region of Venezuela have large populations of HD patients (Simpson *et al.,* 1989; Penney *et al.,* 1990).

3.3 Proteolytic Cleavage of htt

htt is liable to proteolysis by numerous proteases **(Figure 1)**. Initially, htt was recognized as a substrate of caspase and it was the first protein known linked to neurodegenerative disorder which cleaved during apoptosis (Goldberg *et al.,* 1996). Caspases are cysteine-aspartic conserved proteases, associated mainly with apoptotic cell death and vital for the processing of enormous substrates (Orrenius *et al.,* 2003). Fragments generated and processed by caspases are noticeable in brains of HD individual and HD mice before neuronal loss in the region of striatum (Wellington *et al.,* 2000). The efficiency of cleavage depends upon the length of polyQ stretch (Goldberg *et al.,* 1996). Use of site-directed mutagenesis or any other pharmacological approaches to block htt cleavage minimize toxicity in cultured cells (Wellington *et al.,* 2000). Mice expressing a caspase-6 and non-cleavable mutant htt have lesser neurological imperfections as compared with mice having the cleavable mutant htt (Graham *et al.,* 2006). This finding reveals that proteolytic cleavage of the mutant protein through caspases might be a key cause in the toxic events during HD, and that htt works as prosurvival element. htt is also works as substrate for calcium-activated proteases, calpains. Calpains, a cysteine protease normally activated by the elevated levels of intracellular Ca^{2+}, either through the depolarization of plasma membrane or in response to Ca^{2+} discharge from the intracellular storage (Goll *et al.,* 2003). In mice, through overexpression of mutant htt, glutamate release increased from afferent neurons which in turn enhance NMDA-R activity. Enhanced NMDA-R activity increases intracellular Ca^{2+} level and hence calpains get activated in response. Activated calpains cleaves the htt protein in a number of proteolytic products (Gafni *et al.,* 2002) which in turn promote NMDA-R-mediated excitotoxicity (Cowan *et al.,* 2008). Furthermore, calpains can also modulate htt homeostasis through the autophagy. Recent chemical compound and RNAi screenings study in cultured cells reveal that inactivation of calpains possibly activate the autophagy of intracellular aggregates (Miller *et al.,*

2010). It is also shown by RNAi screening study that small fragments of htt can be produced by the proteolytic activity of some matrix metalloproteinases (MMPs) (Williams *et al.*, 2008; Miller *et al.*, 2010). .Decreased MMP activity, particularly MMP-10 and MMP-14, correlates with lesser number of proteolytic fragments and, hence decrease in neuronal degeneration caused by mutant htt in cellular model systems and Drosophila (Miller *et al.*, 2010).

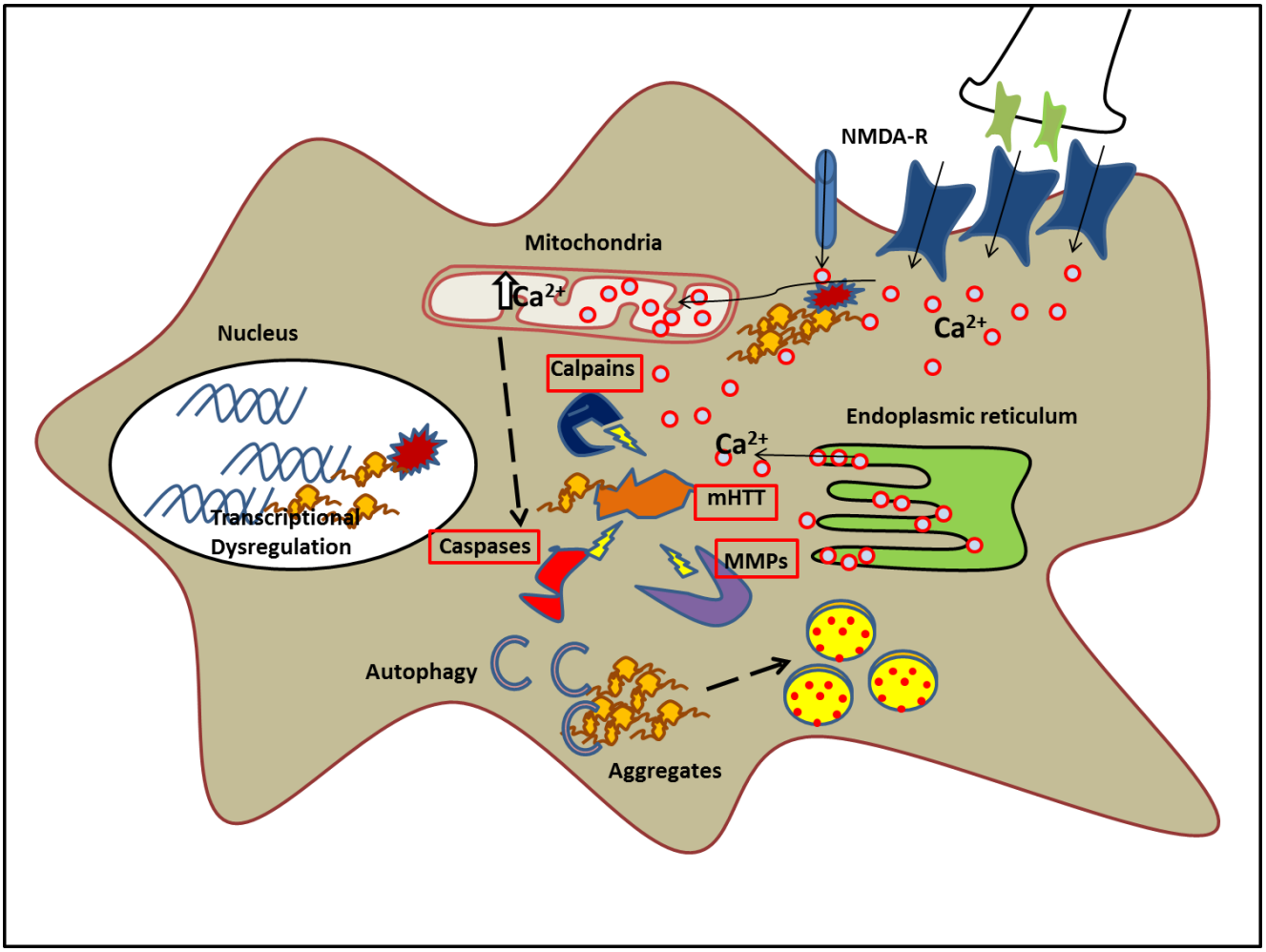


Figure 1: Representation of intracellular events in neurons expressing mutant htt. Processing of mutant htt by caspases, calpains and MMPs and formation of intracellular aggregates

3.4 Diagnosis

Diagnosis of HD is done on the basis of presence of typical motor findings as measured by the Unified HD Rating Scale in the setting of a family history of the disease. Some other manifestations of HD like behavioral and cognitive symptoms are also helpful at the time of presentation or before diagnosis in diagnosis of HD. A DNA test showing abnormal CAG expansion in the htt gene is very common for confirmation of HD in symptomatic individuals. A DNA test can be done in symptomatic individuals under the guidance of veteran clinicians with proper genetic counselling and only at the patient request. Sometimes presymptomatic testing is also performed on the request of patient. Reasons behind presymptomatic testing are financial arrangement, family planning, insurance decisions, and awareness purposes. TRACK-HD, PREDICT-HD (Neurobiological Predictors of Huntington's Disease Trial) and PHAROS (Prospective Huntington at Risk Observational Study) are some of ongoing studies to inspect and detect the HD in people who are gene positive but symptoms are not visible on motor criteria (Biglan *et al.,* 2009; Tabrizi *et al.,* 2009). To identify the biomarkers and understand the sign of onset of disease, there is also a study going on by enrolling and examining individual with HD and their affected and unaffected family members [Cooperative Huntington's Observational Research Trial (COHORT)] (Solomon *et al.,* 2007). The methodology behind the COHORT study is clinical measures and biological samples rather than neuroimaging and anatomical measures. Subtle motor defects have been related with a smaller striatal volume and higher chance of disease diagnosis (Biglan *et al.,* 2009). Lesser scores on the Hopkins Verbal Learning Test-Revised were linked with very close proximity to diagnosis and lesser striatal volumes (Solomon *et al.,* 2007). Individual with an expanded repeat of CAG and preclinical diagnosis of HD also had inaccurate recognition of negative sentiments (Johnson *et al.,* 2007). In addition, self-timed finger tapping, motor exam score, odor identifications, striatal volume, speeded finger tapping and word-list learning in individual in the PREDICT-HD study were all importantly linked with the predicted time to diagnosis (Paulsen *et al.,* 2008). Individual with Expansion reported more psychiatric signs (depression, anxiety) than expansion-negative person (Duff *et al.,* 2007). The numerous motor and non-motor measures on the neurological analysis used to diagnose and TRACK-HD are incorporated in the Unified Huntington's Disease Rating Scale. The Unified rating scale is subdivided into six parts: motor, intellectual, behavioral, and three functional scales (functional capability, functional checklist, and the independence scale). Based on the publication of the American College of Genetics, Individual having forty or more repeats have 100% penetrance (CMG/ASHG statement Laboratory guidelines for Huntington disease genetic testing, 1998). In other words, if individuals have 40 or more number of the gene, they will unpreventably express the disease. Individual with a CAG repeat stretch in the

range of 36–39 would have lesser penetrance with increased probability of expression with longer lifespan of the patient. Although there are some case reports of patients who acquired HD in this range, patients with lesser than 36 repeats of CAG will commonly not manifest HD (Andrich *et al.,* 2008; Kenney *et al.,* 2007). Individuals with size of an allele repeat between 27 to 35 have shown instability in meiosis, predominantly in sperm, showing that the upcoming generation is at greater risk of inheriting an increased number of repeats, increasing the risk of acquiring Huntington's disease. The span of CAG repeats relates usually with the age of onset of HD, but not essentially with the severity or lifespan (Snell *et al.,* 1993). The duration of the disease is commonly 15–20 years, with dystonia, dementia, mutism, and bradykinesia mainly in last-stages. Individual with more dystonia and swallowing problems may have enhanced complications and, therefore, lesser lifespan comparatively. Poor positioning and injury caused by higher amplitude movements could become a safety issue for Chorea. It has been observed that regularly movement may result in head trauma, infections, skin injuries, and even fractures. Problems of immobility, such as infection, pneumonia, skin breakdown, or cardiac disease may result in death. Although, suicide is one of the causes of death in 8-9% of patients and in that percentage of suicide attempt is 25% (Di *et al.,* 1993). Behavioral impairments are severely disabling sign of HD which causes distress to the patient, relatives, family, and caretakers. Stronghold of treatments is cognitive interventions and environmental approaches, but addressing disruptive behaviors can be enhanced by pharmacological agents. Some of the disease like impulsive, anxiety, aggressive, depression and obsessive compulsive behaviors are treated commonly by pharmacologically which require behavioral intervention, but it should be taken care to avoid apathy and over sedation which are common in HD patients. However, it is not clear from the study that for some aspects of the disease pharmacotherapy is less effective than cognitive approaches to treat behavior (Pollard, J. 2008).

3.5 Pharmacological treatment options

There are various agents and surgical techniques have been evaluated in HD for their efficacy on quashing chorea, including dopamine agonists, dopamine-depleting agents, dopamine antagonists, acetylcholinesterase inhibitors, benzodiazepines, glutamate antagonists, antiseizure medications, lithium, cannabinoids, deep brain stimulation, and fetal cell replacement (Adam *et al.,* 2008; Phillips *et al.,* 2008; Roze *et al.,* 2008; Imarisio *et al.,* 2008). Pharmacological interventions usually address the disorders related to hyperkinetic movement associated with HD, such as chorea, myoclonus dystonia, tics and ballism. While choosing the medications, providers need to consider the impact of the agent on psychiatric issues linked with HD, such as irritability, anxiety, cognitive decline. Depression, mania

and apathy associated with HD. Additionally adjunctive therapies, behavioral planning, alternative and complementary therapies and cognitive interventions also play vital role in addressing the symptoms of HD and should be considered while choosing medications. There are so many reviews are available which has explained the symptomatic treatment of HD (Grimbergen *et al.,* 2003; Bonelli *et al.,* 2004; Bonelli *et al.,* 2006; Handley *et al.,* 2006; Nakamura *et al.,* 2007; Adam *et al.,* 2008; Phillips *et al.,* 2008; Roze *et al.,* 2008; Imarisio *et al.,* 2008; Jankovic, J. 2009; Mestre *et al.,* 2009; Frank *et al.,* 2010). Hence, sufficient evidences are not available for long-term symptomatic treatment in HD and double-blind and long-term studies evaluating several treatment strategies in HD are required (Bonelli *et al.,* 2004). On the basis of available evidence, the authors of the Cochrane review revealed that only tetrabenazine (TBZ) exhibited efficacy to ameliorate chorea, but "no declaration can be made about the best medical option for the treatment of motor and non-motor symptoms in HD".

3.5.1 Tetrabenazine

TBZ is the only drug that is approved by US Food and Drug Administration (FDA) for the treatment of chorea associated with HD. TBZ is marketed in many countries like United Kingdom, Denmark, France, Ireland, Germany etc. TBZ depletes dopamine selectively by inhibiting the central VMAT2 (vesicular monoamine transporter type 2) reversibly rather than norepinephrine (Bagchi *et al.,* 1983; Pettibone *et al.,* 1984). TBZ has maximum binding density in the nucleus accumbens, caudate nucleus and putamen, area that is known to be predominantly affected by HD (Mehvar *et al.,* 1987; Thibaut *et al.,* 1995). TBZ reversibly bind to VMAT2 and leads to its last hour monoamine depletion hence VMT2 not modified by long-term treatment (Scherman *et al.,* 1984; Kenney *et al.,* 2007). These important features of the TBZ separate it from the other dopamine-depleting mediator like reserpine. Reserpine binds irreversibly to both VMAT1 and VMAT2, leading to the time of action considerably extended. Furthermore, VMAT2 is localized specially in the central nervous system while VMAT1 is located in to the peripheral nervous system, causing some of adverse effects in the peripheral like orthostatic hypotension, diarrhea etc. Additionally, the two other active metabolites of TBZ, α- and β-dihydrotetrabenazine, have longer half-lives comparatively and are more effectively bound to proteins than the TBZ (Roberts *et al.,* 1981; Roberts et al., 1986; Mehvar *et al.,* 1987). Antichoreic properties and the efficacy of TBZ has convincingly validated in a randomized placebo-controlled, double-blind trial performed by the Huntington Study Group. There are numerous evidences to suggest continuous long-term effectiveness. efficacy and tolerability of TBZ in individual with HD (Jankovic *et al.,* 1997; Kenney *et al.,* 2007; Fasano *et al.,* 2008; Frank, S. 2009). Moreover, drowsiness, agitation, insomnia, akathisia, depressed mood and hyperkinesia are some of adverse events of TBZ that are significantly

more frequent. However, by the completion of the maintenance phase, when subjects were more likely on optimal dosage, significantly, there were no differences found between TBZ and placebo. Hence, it is very important to monitor patients taking TBZ for symptom of depression and suicidal ideation. Although, withdrawal of TBZ causes recurrence of chorea but not worse than before starting the drug (Frank *et al.,* 2007).

3.6. Other antichorea medications

Some of the other medications that are usually considered while treating chorea include dopamine antagonists, glutamate antagonists and benzodiazepines. However, the most commonly considered agents are neuroleptics in the treatment and management of psychosis and chorea in individual with HD. Some of the antipsychotic agents are discussed here, that could be possible drug to treat chorea related to HD

3.6.1 Curcumin

Curcumin, a polyphenol compound, is a plant (Curcumina longa) product and natural inducing agent of HSPs. It has been found that curcumin plays numerous positive effects like in trauma, in vivo models of aging, animal models of certain types of neurodegenerative diseases and ischemia, which make it to highlight among other polyphenol compounds (Al-Omar *et al.,* 2006; Begum *et al.,* 2008; Sharma *et al.,* 2009). It is naturally occurring amyloid binding compound and has been approved by FDA. This biomolecule have the properties of pleiotropic anti-amyloid that propose the possibility for curing different neurodegenerative diseases (Grogan *et al.,* 2013). Oral administration of native curcumin (which is unsulfated and unglucuronidated) easily crosses the blood brain barrier and act as an inhibitor of amyloid aggregation, antioxidant and anti-inflammatory drug (Garcia-Alloza *et al.,* 2007). Plaque burden is reduced when curcumin and related curcuminoids is administered in AD model. It also protects against Aβ-toxicity and hence improves cognitive function (Garcia *et al.,* 2007; Hickey *et al.,* 2012). Furthermore, treatment of CAG140 KI mice with curcumin diminished neuropathology and transcriptional shortages, including drop in levels of mutant htt aggregates (Hickey *et al.,* 2012).

3.6.2 Berberine

Berberine (BBR) is the plant-derived protoberberine alkaloid. It is obtained from the bark and roots of many plants like Berberissp, Coptis chinenses and has been used for over sixty years in China to cure bacterial diarrhea (Takase *et al.,* 1993; Kong *et al.,* 2004). However, Current discoveries have shown a plenty of additional uses for this natural compound, which includes its ability to combat diabetes cardiac disease, hypercholesterolemia, inflammation, and the side-effects of radiotherapy (Iizuka *et al.,* 2000; Hayashi *et al.,* 2007; Zhang *et al.,* 2010; Kwon *et al.,* 2010; Jiang *et al.,* 2011; Dong *et al.,* 2012; Kim *et al.,* 2014; Shin *et al.,* 2014). High tolerance for orally taken doses makes it safer for long-term uses. BBR is freely found in the bloodstream for more than two hours after oral intake and it is able to freely cross the blood-brain-barrier (Wang *et al.,* 2005; Jiang *et al.,* 2011; Lan *et al.,* 2014), which make it an ideal drug candidate to test its protective effects on chronic neurological disorders like AD, PD and HD. The most promising and relevant discovery to BBR's ability to reduces the symptoms and pathology associated with Parkinson's disease (PD) and Alzheimer's Disease (AD) in animal models shown the promising hope that it could do the same against HD, as both diseases are instigated by the accretion of misfolded proteins (Wang *et al.,* 2005; Zhu *et al.,* 2006; Asai *et al.,* 2007; Panahi *et al.,* 2013). Here, we examined the effects of BBR on mutant htt accumulation and toxicity through Bioinformatics approach.

3.6.3 Aripiprazole

Aripiprazole is a peculiar antipsychotic drug with partial agonist properties at dopamine (DA) D2/D3 receptors and lesser side-effect property. Due to its efficacy and low side-effect profile, aripiprazole has been recognized as one of the best option for treatment of schizophrenia (DeLeon *et al.,* 2004; Lieberman *et al.,* 2004) and bipolar disorder (Keck *et al.,* 2003). In Comparison of other antipsychotic drugs, aripiprazole is normally safe and well tolerated by patients, rarely inducing extrapyramidal and metabolic adverse effects when used in schizophrenic individual (Kane *et al.,* 2002; Pigott *et al.,* 2003) and produces lesser side effects, including less extrapyramidal symptoms, reduced incidence of sedation and weight gain, and a negligible risk for diabetes and hyperlipidemia (DeLeon *et al.,* 2004).Owing to its atypical neuropharmacological profile, aripiprazole might be an interesting novel symptomatic option that could be used in combination with other drugs such as tetrabenazine which acts on chorea to ameliorate the symptoms of HD.

3.6.4 Clozapine

Clozapine, a tricyclic dibenzodiazepine is commonly used antipsychotic drug in the treatment of schizophrenia. It is predominantly useful for the individual intolerant to the side effects of traditionally used antipsychotics (Kane *et al.,* 1988). Although clozapine may cause agranulocytosis in some patients, the incidence is almost 0.37%. Additionally, clozapine does not cause severe extrapyramidal toxicity or permanent neurologic side effects (Lieberman *et al.,* 1998). Because its usefulness outweighs its side effects, clozapine has been recognized globally as an antipsychotic drug and commonly used to treat around 31.7% of Chinese schizophrenia patients (Si *et al.,* 2010). Norclozapine, a pharmacologically active metabolite produced when clozapine metabolized by human cytochrome P450 (CYP) isozyme 1A2 (Bertilsson *et al.,* 1994). Many scientists have shown that the degree to which clozapine is changed into norclozapine predicts the medical consequence with respect to multiple events of cognition, positive and negative symptoms, as well as quality of life (Flanagan *et al.,* 2003; Mauri *et al.,* 2003). Thus, clozapine and its pharmacological products, norclozapine could be promising therapeutic approach towards HD.

3.6.5 Olanzapine

Olanzapine is a U.S. FDA approved drug, used to cure schizophrenia and bipolar disorder. It is structurally very similar to clozapine and quetiapine.

3.6.6 Pridopidine

Pridopidine stabilize DA and belongs to dopamine's family. On the basis of prevailing dopaminergic tone, pridopidine regulates and modulates DA transmission and also control the regulation of both hypoactive and hyper functioning (Ponten *et al.,* 2010). Pridopidine has been successfully tested in individual with PD (Tedroff *et al.,* 2004), schizophrenia (Carlsson *et al.,* 2006) and is presently used in development of drug for the treatment of HD patient. Recent clinical studies show that pridopidine has a promising therapeutic strength for patients with HD (Yebenes *et al.,* 2001; Squitieri *et al.,* 2013).

3.6.7 Quetiapine

Quetiapine is an unusual FDA approved neuroleptic with a distinct pharmacological profile from typical neuroleptics that acts via blocking dopamine D_2 receptors. In USA, it is currently used for the treatment of patients suffering from schizophrenia which is a major disorder characterized by bipolar I disorder and depression. Furthermore, it is frequently prescribed off-label for obsessive-compulsive disorder, anxiety, depression and sleep disturbance (Bowden *et al.,* 2005; McIntyre *et al.,* 2007;

McIntyre *et al.,* 2007).Thus, in addition to D_2 antagonistic properties, quetiapine seems to have different modes of action. quetiapine has an additional distinctive profile with a strong affinity for histamine (H_1) and α1-adrenergic receptors among the atypical neuroleptics that strongly bound to serotonin (5-HT_{2A}) but a relatively weakly bound to dopamine (D_2) receptors (Mohr *et al.,* 2002; Nemeroff *et al.,* 2002). On the basis of its distinctive features, we hypothesized that quetiapine would have unique pharmacological actions and functions through different pathways as compare to the tradional neuroleptics and hence it could be a novel drug for treatment of HD.

3.6.8 Rilmenidine

Rilmenidine is an antihypertensive agent which acts on α_2-adrenoceptors and imidazoline I_1 receptors in the brain and in the periphery (Harron *et al.,* 1995). It acts primarily within the rostral part of the ventrolateral medulla to reduce sympathic outflow to peripheral organs (Montastruc *et al.,* 1989). It is 30 times more selective for imidazoline I_1 receptors than for α_2-adrenoceptors in comparison to the prototypical compound clonidine and thus causes fewer adverse central side effects. This drug is like sedation or antinociception (Chan *et al.,* 1996; Kamisaki *et al.,* 1990). It is known that rilmenidine goes to the brain and performs its antihypertensive function (Safar *et al.,* 1989). The imidazoline I_1 receptor is expressed in HD affected region in both the rodent and human, including the striatum, hippocampus cerebral cortex, hypothalamus and ventrolateral medulla (Vos *et al.,* 1994). As rilmenidine is safer comparatively, it could be considered for the treatment of HD individual.

3.6.9 Tiapride

Tiapride is an atypical antipsychotic agent. It is a selectively block dopamine D2-receptor with little side effects like catalepsy and sedation. It shows better activity at receptors previously sensitized to dopamine and those located extra-striatally. Tiapride exhibits antidyskinetic activity due to its antidopaminergic actions, and it also has anxiolytic activity but mechanisms involved is not understood.

4. MATERIALS AND METHODS

4.1 Retrieval, Visualization and quality assessment of Huntingtin (htt) protein

To visualize the 3D structure of the Huntington disease related protein Huntingtin (htt) with PDB ID: 3IOW was identified using Protein Data Bank (PDB). Once the PDB ID of protein was identified, it was visualized using pymol. RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) was used for visualizing the Ramachandran Plot of protein for the structural evaluation and stereo chemical analysis of proteins.

4.2 Ligand Optimization

Sdf files of ligands along with their physical and chemical properties were retrieved from PubChem Compound Database (http://www.ncbi.nlm.nih.gov/pccompound).These sdf files converted into pdb format with the help OpenBabel tool.

4.3 Lipinski Filter Analysis of Screened Drugs (Biomolecules)

Another online tool Sanjeevini (http://www.scfbio-iitd.res.in/sanjeevini/sanjeevini.jsp) is used to get the information of drugs with the help of Lipinski Rule.

Lipinski rule (or Lipinski rule of five) helps to differentiate drug and nondrug like molecules. It is used to identify the possibility of success or failure due to drug likeness for molecules fulfilling with two or more of the following rules

a) Molecular mass should be<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.

e) Molar refractivity should be between 40 -130.

4.4 Active Site Prediction

Castp server (http://www.sts.bioe.uic.edu/castp/) was used to predict the active sites of protein. Castp could also be used to measure area, circumference of mouth openings of each binding site in solvent and molecular accessible surface. PDB file of protein was uploaded in the server and it showed the

ligand binding sites present in protein and the most conserved site was selected and all the amino acid residues involved in binding with ligands were retrieved.

4.5 Preparation of Protein and ligand molecules

Preparation of protein involves the addition of polar hydrogen atoms, neutralization of charge and removal of any miscellaneous structures from the protein molecule by Autodock 4.2.1 whereas ligand preparation involves the neutralization of charge.

4.6 Docking Study

Prepared and optimized structures of ligands and protein were ultimately used for molecular docking using Autodock 4.2.1 for predicting the possible protein–ligand interactions and the results that include the understanding of the association that involves H-bonding and hydrophobic interactions were analyzed using LIGPLOT1.4.5, a program to generate schematic diagrams of protein-ligand interactions.

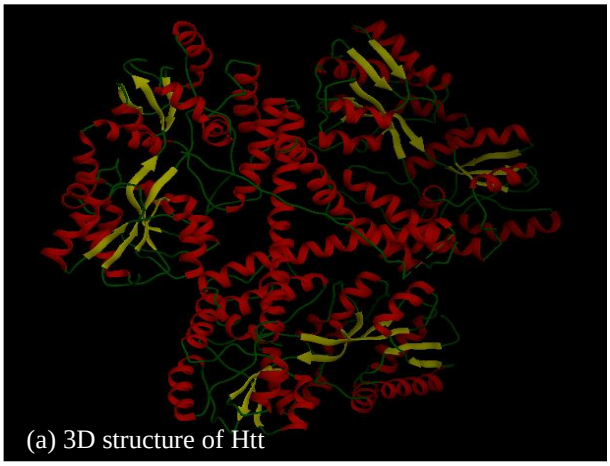
5. RESULTS AND DISCUSSION

5.1 Visualization and quality assessment of Huntingtin (htt) protein

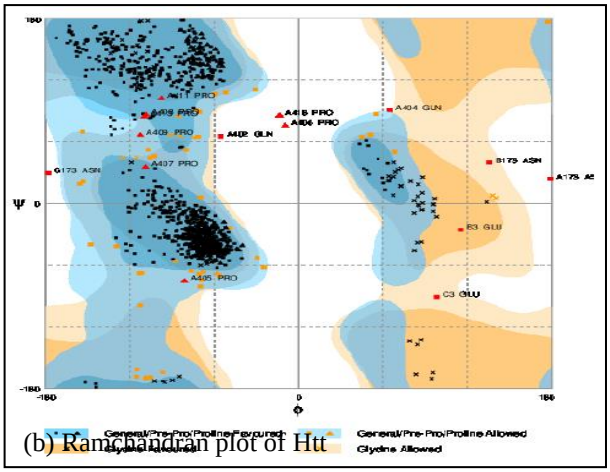
3D structure of htt protein (PDB ID-3IOW) was obtained through pymol **[Figure-2 (a)]**. There were no steric hindrances found in the structure generated. Further, Ramachandran Plot was obtained through RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) to validate the reliability of predicted 3D structure of htt protein **[Figure-2 (b)]**. It examined the stereo chemical quality of a protein structure by analyzing geometry of residues as well as overall structure geometry **[Figure-2 (b)]**. RAMPAGE showed 94.7% of residues in the most favorable regions, 4.1% residues in additionally allowed and 1.2% outlier regions. Furthermore, Errat server calculated overall 89.114% accuracy of htt protein.

5.2 Active site prediction

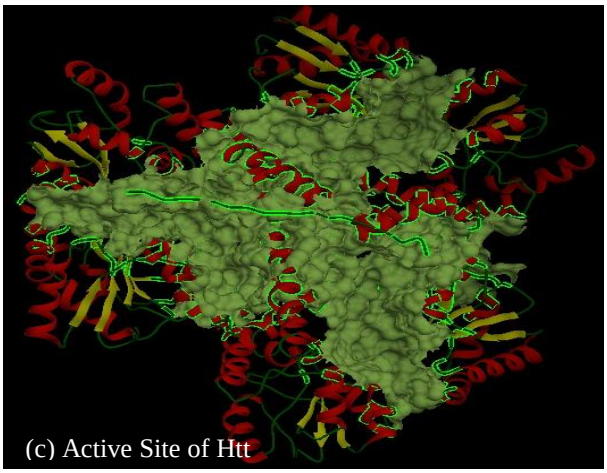
CastP server (http://www.sts.bioe.uic.edu/castp/) was used to identify the active site of htt protein where the ligand binds effectively. This server calculates the possible active sites from the 3D atomic coordinates of the protein. Active site identification is very useful for determination of potential sites for binding of ligand to protein in molecular docking. Residues of active site responsible for binding ligand, site volume and protein volume for 155 active sites for htt were predicted. Among the 155 active sites, 155th site was highly conserved **[Figure-2 (c)]**. Residues found in 155th active site which interact to ligand at the time of molecular docking are ILE^{11}, ASN^{12}, ASP^{14}, LYS^{15}, GLY^{16}, PRO^{40}, LYS^{42}, LEU^{43}, GLU^{44}, GLU^{45}, PRO^{48}, GLN^{49}, ALA^{52}, THR^{53}, PHE^{61}, TRP^{62}, ALA^{63}, HIS^{64}, ASP^{65}, ARG^{66}, GLY^{69}, TYR^{70}, ALA^{71}, GLN^{72}, SER^{73}, GLY^{74}, LEU^{75}, LEU^{76}, ALA^{77}, GLU^{78}, ARG^{98}, TYR^{99}, ASN^{100}, LYS^{102}, ILE^{104}, ALA^{109}, VAL^{110}, GLU^{111}, ASN^{150}, GLU^{153}, PRO^{154}, TYR^{155}, PHE^{156}, TYR^{167}, PHE^{169}, TYR^{171}, GLY^{174}, LYS^{175}, TYR^{176}, ASP^{177}, LYS^{179}, ASP^{180}, VAL^{181}, GLY^{182}, ASP^{184}, ASN^{185}, ALA^{186}, LYS^{189}, TYR^{210}, PRO^{229}, TRP^{230}, TRP^{232}, SER^{233}, ASP^{236}, GLY^{260}, VAL^{261}, LEU^{262}, ALA^{268}, ASN^{294}, LYS^{297}, PRO^{298}, LEU^{299}, GLY^{300}, LEU^{311}, ARG^{316}, ILE^{317}, MET^{330}, PRO^{331}, ASN^{332}, ILE^{333}, PRO^{334}, GLN^{335}, MET^{336}, SER^{337}, ALA^{338}, PHE^{339}, TRP^{340}, TYR^{341}, ALA^{342}, ARG^{344}, THR^{345}, ALA^{346}, ILE^{348}, ASN^{349}, ARG^{345}, GLN^{355}, ASP^{358}, ALA^{359}, ALA^{360}, ALA^{362}, ALA^{363}, ALA^{364}, GLN^{365}, THR^{366}, ASN^{367}, ALA^{368}, ALA^{369}, ALA^{370}, MET^{371}, ALA^{372}, THR^{373}, LEU^{374}, GLU^{375}, LYS^{376}, LEU^{377}, MET^{378}, LYS^{379}, ALA^{380}, PHE^{381}, GLU^{382}, SER^{383}, LEU^{384}, LYS^{385}, SER^{386}, PHE^{387}, GLN^{388}, GLN^{389}, GLN^{390}, GLN^{391}, GLN^{401}, PRO^{406}, PRO^{408}, PRO^{409}, PRO^{410}, PRO^{411}, PRO^{412}, PRO^{413}, PRO^{414}, PRO^{415}, GLN^{416}, LEU^{417}, PRO^{418} and GLN^{419}.



(a) 3D structure of Htt



(b) Ramchandran plot of Htt



(c) Active Site of Htt

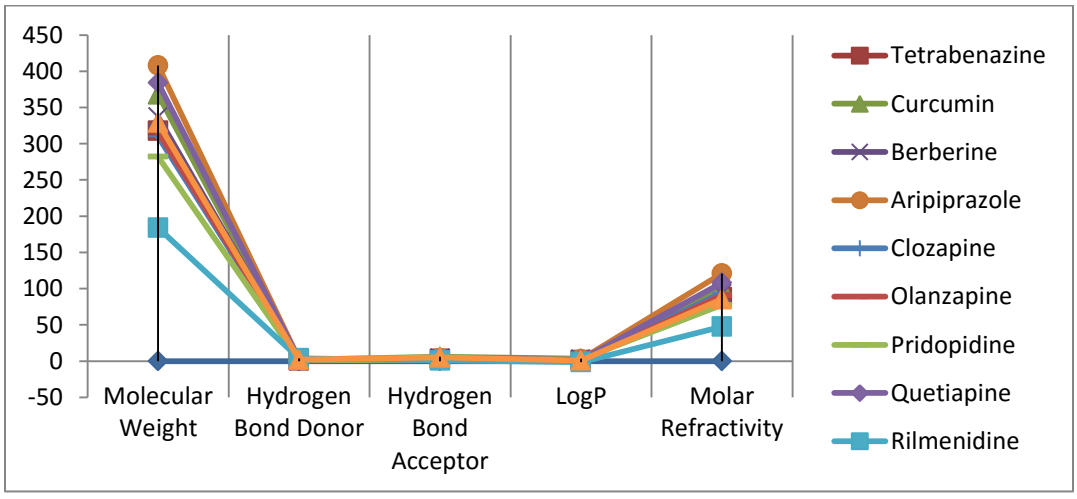
Figure 2: 3D structures, Ramachandran Plot and Active site of Huntingtin (htt). Generated model had no steric clashes and its most conserved active site is located in the hydrophobic region of Huntingtin (htt) protein.

5.3 Lipinski filter analysis of screened drugs

All the biomolecules screened on the basis of Lipinski filter analysis. Values of different parameters of Lipinski filter are given in table (**Table-1**). Analysis for drug likeness is done by drawing the graph (**Graph-1**). Lipinski filter analysis revealed that the all these nine experimental biomolecules act as drug on the basis of Lipinski rule of five. When it was analyzed that all screened biomolecules had drug like property, these were then used for docking purposes to understand the interaction of proteins and the screened drug molecule.

Biomolecules	Molecular Weight	Hydrogen Bond Donor	Hydrogen Bond Acceptor	LogP	Molar Refractivity
Tetrabenazine (**Control**)	318	1	3	1.588	88.323
Curcumin	368	2	6	3.369	102.01
Berberine	337	0	4	2.732	93.033
Aripiprazole	408	2	4	2.519	120.88
Clozapine	310	3	2	1.34	93.74
Olanzapine	316	3	1	-0.277	90.83
Pridopidine	282	1	2	2.34	77.42
Quetiapine	384	2	4	1.43	108.24
Rilmenidine	184	4	1	-1.38	47.69
Tiapride	329	2	5	0.834	85.39

Table 1:-Calculation of different parameter on the basis of Lipinski Rule



Graph 1: Comparison between different drugs on the basis of Lipinski Rule

5.4 Docking calculation of Biomolecules with huntingtin (htt)

Biomolecule	Est. Free Energy of Binding	Est. Inhibition Constant	Est. Intermolecular Energy	vdW+Hbond+desolv Energy	Electrostatic Energy	Est. Internal Energy	Torsional Free Energy
Tetrabenazine (**Control**)	-5.17 (kcal/mol)	161.18 µM	-7.26 (kcal/mol)	-6.45 (kcal/mol)	-0.82 (kcal/mol)	-0.54 (kcal/mol)	+2.09 (kcal/mol)
Curcumin	-6.30 (kcal/mol)	24.30 µM	-8.38 (kcal/mol)	-8.19 (kcal/mol)	-0.20 (kcal/mol)	-2.20 (kcal/mol)	+2.09 (kcal/mol)
Berberine	-5.29 (kcal/mol)	132.32 µM	-8.22 (kcal/mol)	-7.75 (kcal/mol)	-0.47 (kcal/mol)	-0.91 (kcal/mol)	+2.09 (kcal/mol)
Aripiprazole	-6.14 (kcal/mol)	31.81 µM	-8.22 (kcal/mol)	-7.75 (kcal/mol)	-0.47 (kcal/mol)	-0.91 (kcal/mol)	+2.09 (kcal/mol)
Clozapine	-5.29 (kcal/mol)	133.41 µM	-7.37 (kcal/mol)	-6.77 (kcal/mol)	-0.60 (kcal/mol)	-0.42 (kcal/mol)	+2.09 (kcal/mol)
Olanzapine	-5.09 (kcal/mol)	184.52 µM	-7.18 (kcal/mol)	-6.20 (kcal/mol)	-0.98 (kcal/mol)	-0.29 (kcal/mol)	+2.09 (kcal/mol)
Pridopidine	-6.31 (kcal/mol)	23.87 µM	-8.39 (kcal/mol)	-7.62 (kcal/mol)	-0.78 (kcal/mol)	-0.41 (kcal/mol)	+2.09 (kcal/mol)
Quetiapine	-6.03 (kcal/mol)	37.97 µM	-8.12 (kcal/mol)	-6.95 (kcal/mol)	-1.17 (kcal/mol)	-1.60 (kcal/mol)	+2.09 (kcal/mol)
Rilmenidine	-3.57 (kcal/mol)	2.40 µM	-5.66 (kcal/mol)	-5.63 (kcal/mol)	-0.03 (kcal/mol)	-0.47 (kcal/mol)	+2.09 (kcal/mol)
Tiapride	-5.69 (kcal/mol)	67.83 µM	-7.78 (kcal/mol)	-6.85 (kcal/mol)	-0.92 (kcal/mol)	-1.36 (kcal/mol)	+2.09 (kcal/mol)

Table 2: Docking calculation of Biomolecule with Huntingtin (htt) gene

5.4.1 Huntingtin (htt) interaction with Tetrabenazine

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -5.17 kcal/mol., Est. Inhibition Constant, Ki is 161.18 µM and Intermolecular Energy is -7.26 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -6.45 kcal/mol. and -0.82 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -.54 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(a)]**.

5.4.2 Huntingtin (htt) interaction with Curcumin

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -6.30 kcal/mol., Est. Inhibition Constant, Ki is 24.30 µM and Intermolecular Energy is -8.38 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -8.19 kcal/mol. and -0.20 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -

2.20 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(b)]**.

5.4.3 Huntingtin (htt) interaction with Berberine

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -5.29 kcal/mol., Est. Inhibition Constant, Ki is 132.32 μM and Intermolecular Energy is -8.22 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -7.75 kcal/mol. and -0.47 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -.91 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(c)]**.

5.4.4 Huntingtin (htt) interaction with Aripiprazole

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -6.14 kcal/mol., Est. Inhibition Constant, Ki is 31.81 μM and Intermolecular Energy is -8.22 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -7.75 kcal/mol. and -0.47 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -.91 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(d)]**.

5.4.5 Huntingtin (htt) interaction with Clozapine

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -5.29 kcal/mol., Est. Inhibition Constant, Ki is 133.41 μM and Intermolecular Energy is -7.37 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -6.77 kcal/mol. and -0.60 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -.42 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(e)]**.

5.4.6 Huntingtin (htt) interaction with Olanzapine

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -5.09 kcal/mol., Est. Inhibition Constant, Ki is 184.52 μM and Intermolecular Energy is -7.18 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -6.20 kcal/mol. and -0.98 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -

.29 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(f)]**.

5.4.7 Huntingtin (htt) interaction with Pridopidine

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -6.31 kcal/mol., Est. Inhibition Constant, Ki is 23.87 μM and Intermolecular Energy is -8.39 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -7.62 kcal/mol. and -0.78 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -.41 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(g)]**.

5.4.8 Huntingtin (htt) interaction with Quetiapine

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -6.03 kcal/mol., Est. Inhibition Constant, Ki is 37.97 μM and Intermolecular Energy is -8.12 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -6.95 kcal/mol. and -1.17 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -1.60 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(h)]**.

5.4.9 Huntingtin (htt) interaction with Rilmenidine

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -3.57 kcal/mol., Est. Inhibition Constant, Ki is 2.40 μM and Intermolecular Energy is -5.66 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -5.63 kcal/mol. and -0.03 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -.47 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(i)]**.

5.4.10 Huntingtin (htt) interaction with Tiapride

Docking study of tetrabenazine with huntingtin (htt) shows that Free energy of binding of tetrabenazine with huntingtin gene is -5.69 kcal/mol., Est. Inhibition Constant, Ki is 67.83 μM and Intermolecular Energy is -7.78 kcal/mol. VdW+ Hbond + desolv Energy and Electrostatic Energy are -6.85 kcal/mol. and -0.92 kcal/mol. respectively. Total Internal Energy and Torsional Free Energy are -

1.36 kcal/mol. and 2.09 kcal/mol. respectively (**Table: 2**). Interaction pattern of docking study between htt and tetrabenazine has been shown in **[Figure-3-(j)]**.

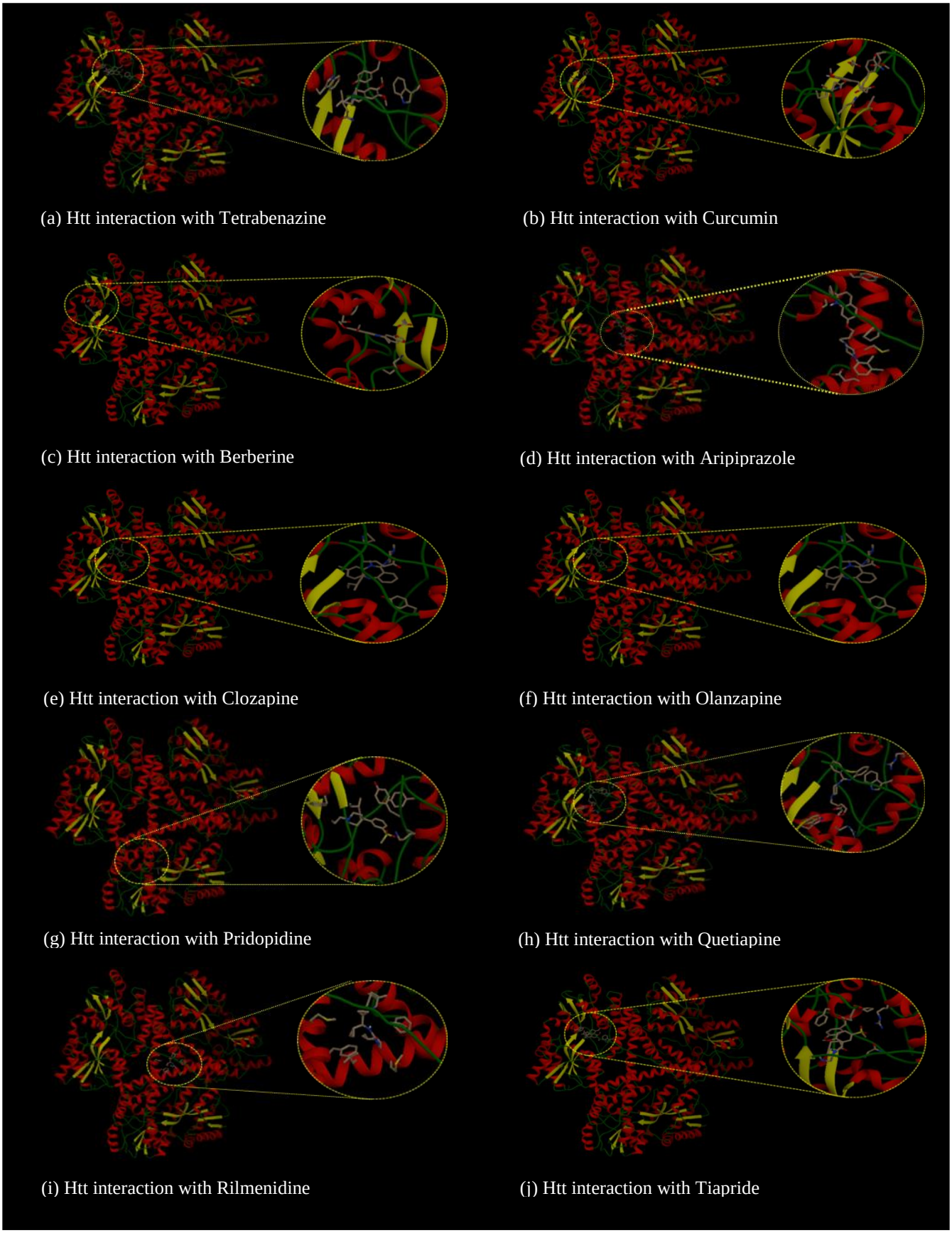


Figure 3: Docking study of huntingtin (htt) with selected Biomolecules

5.5 Interacting residue of huntingtin (htt) with Biomolecules

Ligand and protein analysis revealed that tetrabenazine forms hydrophobic binding with GLU^{111}, GLU^{153}, TRP^{155}, TRP^{220}, MET^{330}, TRP^{340}, PRO^{418}, GLN^{419} residues **[Figure 4-(a)]** of htt gene. Curcumin forms H-bonding with ASN^{12}, LYS^{15}, LEU^{299} , PRO^{418} residues and hydrophobic interaction with ASN^{12}, ASP^{14}, LYS^{15}, TRP^{62}, ALA^{63}, ALA^{109}, GLN^{111}, TRP^{230}, GLY^{260}, LEU^{262}, GLN^{416}, LEU^{417}, PRO^{418}, GLN^{419} residues **[Figure 4-(b)]** of htt gene. Berberine forms hydrophobic interaction with LYS^{13}, ALA^{109}, VAL^{110}, PRO^{229}, TRP^{230}, TRP^{232}, SER^{233}, GLY^{260}, LEU^{262}, PRO^{298}, LEU^{299}, GLY^{300}, ARG^{316} residues **[Figure 4-(c)]** of htt gene. Aripiprazole forms hydrophobic interaction with SER^{337}, TYR^{341}, MET^{370}, GLU^{375}, GLU^{382}, PRO^{411}, PRO^{412}, PRO^{414} residues [**Figure 4-(d)]** of htt gene. Clozapine forms hydrophobic interaction with ALA^{162}, GLY^{165}, GLY^{166}, TYR^{167}, PHE^{169}, TYR^{171}, TYR^{176}, LYS^{256}, GLN^{325}, GLY^{327}, GLU^{328}, ILE^{329} residues **[Figure 4-(e)]** of htt gene. Olanzapine forms hydrophobic interaction with GLU^{111}, GLU^{153}, TYR^{155}, PHE^{156}, TRP^{230}, GLY^{260}, PRO^{418} residues **[Figure 5-(a)]** of htt gene. Pridopidine forms H-bonding with TYR^{171} and hydrophobic interaction with PHE^{92}, ASP^{95}, TYR^{167}, PHE^{169}, LYS^{170}, TYR^{176}, ALA^{324}, GLN^{325}, GLY^{327}, GLU^{328}, ILE^{329} residues **[Figure 5-(b)]** of htt gene. Quetiapine forms H-bonding with ARG^{66} and hydrophobic interaction with GLU^{44}, ALA^{63}, ASP^{65}, GLU^{153}, TYR^{155}, PHE^{156}, MET^{330}, TRP^{340}, ARG^{344}, GLN^{416}residues **[Figure 5-(c)]** of htt gene. Rilmenidine forms H-bonding with PRO^{406} and hydrophobic interaction LEU^{374}, LEU^{377}, MET^{378}, PHE^{381}, PRO^{408} residues **[Figure 5-(d)]** of htt gene. Tiapride forms H-bonding with ARG^{344} and hydrophobic interaction GLU^{153}, PRO^{154}, TYR^{155}, PHE^{156}, TRP^{230}, TRP^{340}, GLN^{416}, LEU^{417}, PRO^{418} residues **[Figure 5-(e)]** of htt gene.

Biomolecule	Interacting residues
Tetrabenazine (**Control**)	GLU^{111}, GLU^{153}, TRP^{155}, TRP^{220}, MET^{330}, TRP^{340}, PRO^{418}, GLN^{419}
Curcumin	ASN^{12}, ASP^{14}, LYS^{15}, TRP^{62}, ALA^{63}, ALA^{109}, GLN^{111}, TRP^{230},GLY^{260}, LEU^{262}, GLN^{416}, LEU^{417}, PRO^{418}, GLN^{419}
Berberine	LYS^{13}, ALA^{109}, VAL^{110}, PRO^{229}, TRP^{230}, TRP^{232}, SER^{233}, GLY^{260}, LEU^{262}, PRO^{298}, LEU^{299}, GLY^{300}, ARG^{316}
Aripiprazole	SER^{337}, TYR^{341} , MET^{370}, GLU^{375}, GLU^{382}, PRO^{411}, PRO^{412}, PRO^{414}
Clozapine	ALA^{162}, GLY^{165}, GLY^{166}, TYR^{167} , PHE^{169}, TYR^{171}, TYR^{176}, LYS^{256}, GLN^{325}, GLY^{327}, GLU^{328}, ILE^{329}
Olanzapine	GLU^{111}, GLU^{153}, TYR^{155}, PHE^{156}, TRP^{230}, GLY^{260}, PRO^{418}
Pridopidine	PHE^{92}, ASP^{95}, TYR^{167}, PHE^{169},LYS^{170} , TYR^{176}, ALA^{324}, GLN^{325}, GLY^{327}, GLU^{328}, ILE^{329}
Quetiapine	GLU^{44}, ALA^{63}, ASP^{65}, GLU^{153}, TYR^{155}, PHE^{156}, MET^{330}, TRP^{340}, ARG^{344}, GLN^{416}
Rilmenidine	LEU^{374}, LEU^{377}, MET^{378}, PHE^{381}, PRO^{408}
Tiapride	GLU^{153}, PRO^{154}, TYR^{155}, PHE^{156}, TRP^{230}, TRP^{340}, GLN^{416}, LEU^{417}, PRO^{418}

Table 3: Interacting residues of Huntingtin (htt) gene with biomolecule

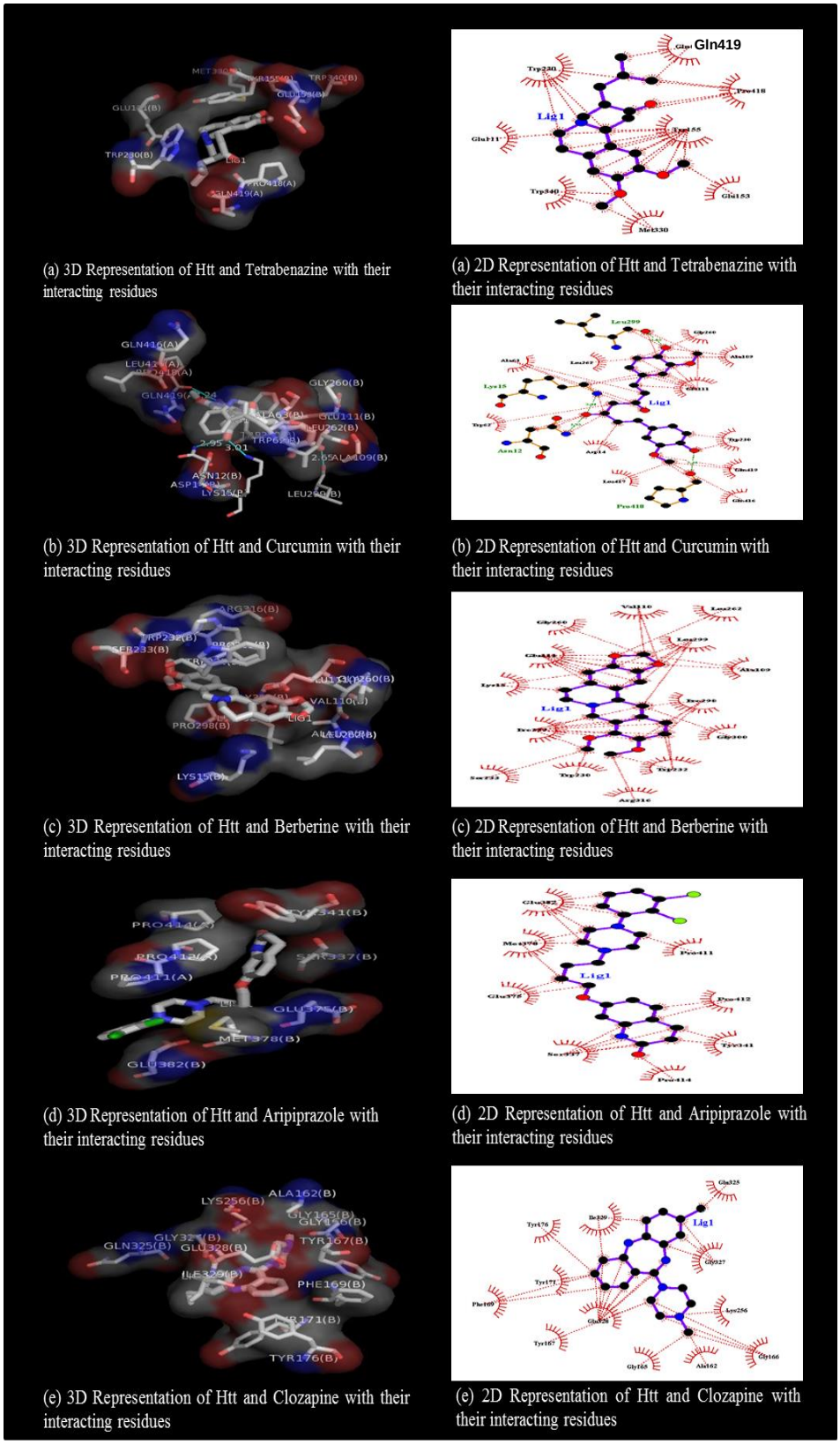


Figure 4: Binding site of htt with selected compounds. 3D and 2D pattern of protein-ligand interaction shows the interacting residues of Huntingtin (htt) ligand binding.

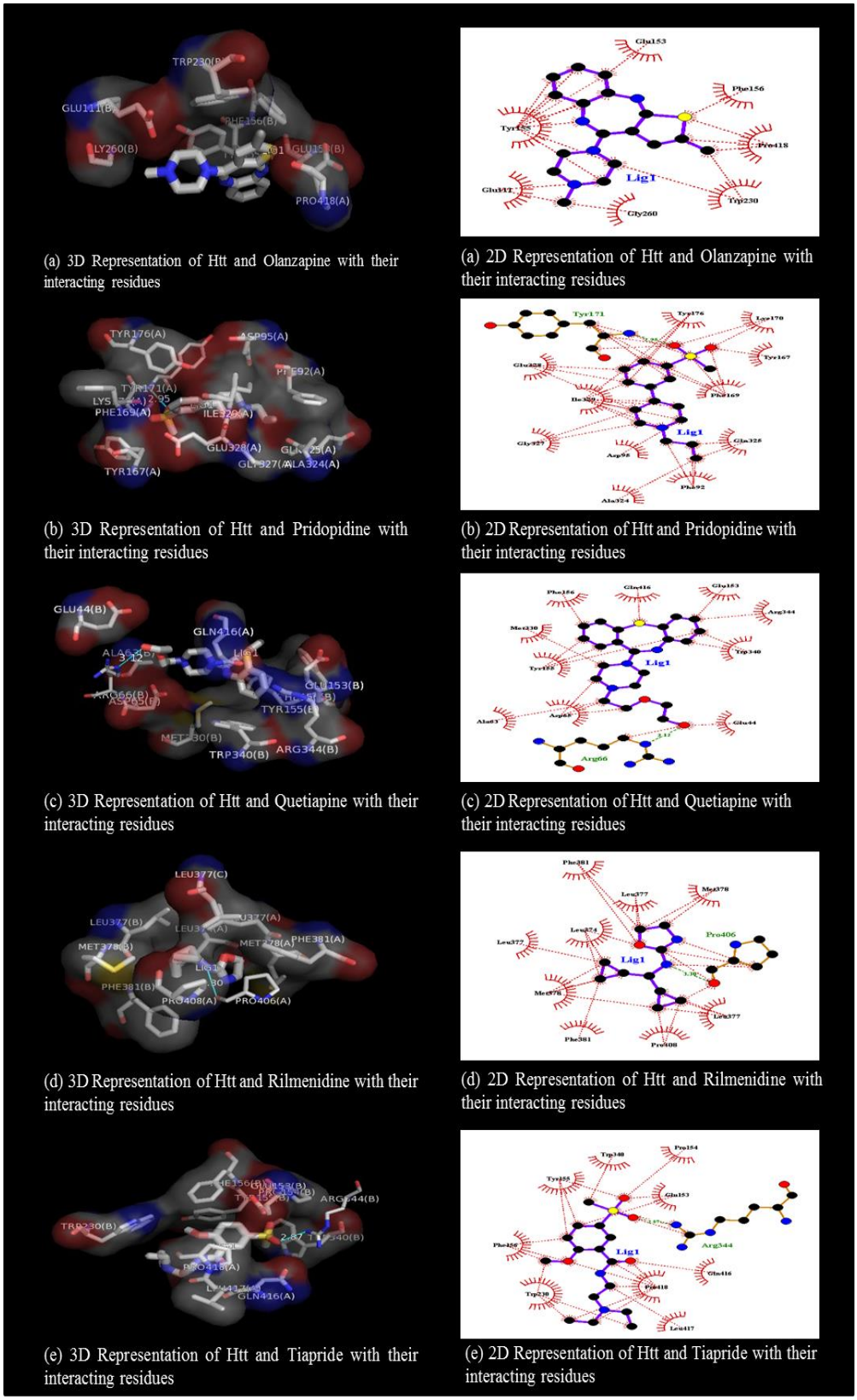


Figure 5: Binding site of htt with selected compounds. 3D and 2D pattern of protein-ligand interaction shows the interacting residues of Huntingtin (htt) ligand binding.

6. CONCLUSION

Recent therapeutics advancement in HD reveals the promising role of biomolecules that has been shown to be potent antipsychotic agents for the treatment of HD. 3D structure of htt was checked for the presence of steric clashes and its suitability in the docking procedure using Ramachandran plot prediction analysis via RAMPAGE. RAMPAGE showed 94.7% of residues in the most favorable regions, 4.1% residues in additionally allowed and 1.2% outlier regions which shows that stereo chemical quality of protein structure is good. Furthermore, Errat server calculated overall 89.114% accuracy of htt protein. Among one hundred fifty five binding sites obtained from CastP Server for huntingtin, site 155 was highly conserved within all the binding sites of htt protein. Active site identification is very useful for determination of potential sites for binding of ligand to protein in molecular docking. Nine natural products like Curcumin, Berberine, Aripiprazole, Clozapine, Olanzapine, Pridopidine, Quetiapine, Rilmenidine and Tiapride were selected for molecular docking purposes and compared with docking analysis of FDA approved drug tetrabenazine.

Lipinski Filter Investigation of all the natural compounds showed that all the compounds had drug likeness but Rilmenidine had more drug likeness followed by Pridopidine and Aripiprazole had least drug likeness. Molecular Docking study showed that all the nine natural biomolecules are interacting with the identified active site. Furthermore, comparison of binding atomic coordination with the template complex coordination revealed that docked drug coordination was similar with the known coordination. Inhibition Constant (Ki) of Curcumin, Berberine, Aripiprazole, Clozapine, Olanzapine, Pridopidine, Quetiapine, Rilmenidine and Tiapride for htt was found to be 24.30 µM, 132.32 µM, 31.81 µM, 133.41 µM, 184.52 µM, 23.87 µM, 37.87 µM, 2.40 µM and 67.83 µM while the inhibition constant for Tetrabenazine (FDA approved drug for HD) was found to be 161.1824.30 µM, 132.32 µM, 31.81 µM, 133.41 µM, 184.52 µM, 23.87 µM, 37.87 µM, 2.40 µM and 167.83 µM. it suggests that all the selected natural biomolecules are effective against htt (**Table 2)**.Curcumin, Berberine, Aripiprazole, Clozapine, Olanzapine, Pridopidine, Quetiapine, Rilmenidine and Tiapride bind to the active site of mutant htt protein. Finally, docking analysis revealed that Curcumin, Berberine, Aripiprazole, Clozapine, Pridopidine, Quetiapine and Tiapride were more effective as compared with Tetrabenazine (control) as these compounds had lower Inhibition Constant, Ki and free energy of binding in comparison with Tetrabenazine (Park, H., 2006).

The results can be validated through laboratory trials and clinical trials of the drugs as one of future perspective options.

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