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Chrysin Interaction with the Blood Brain Barrier Disrupting Proteins in Autism – An *in Silico* Perspective

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ABSTRACT:

Autism spectrum disorder (ASD) is a neuro developmental problem associated with deficiencies in social skills. The loss of the blood–brain barrier (BBB) integrity is linked to the onset and aggravation of a multitude of neurodegenerative and neuropsychiatric ailments including ASD. Chrysin is a bioflavonoid with multifaceted therapeutic efficacy. The main aim of the study was to investigate the therapeutic role of chrysin in Autism with respect to the Blood brain barrier disruption (BBB). The interaction of chrysin with BBB-disrupting proteins was carried out via *in silico* method. The interactions of Chrysin against Resistin (1RFX), IL-1 (1T4Q), TNF (1TNF), VCAM-1 (1VSC), and MMP-9 (6ESM) were studied using molecular docking to unravel the therapeutic efficacy of chrysin against these BBB disrupting proteins in which risperidone was used as a control. Pharmacokinetic studies revealed that chrysin has strong BBB and CNS permeability followed by drug likeness. Among all the targets MMP-9 had higher binding affinity to both chrysin and risperidone. The nature of bonds interacting in molecular docking is Vanderwaals forces, Hydrogen bond, Pi- anion and carbon hydrogen bonds. The formation of stable hydrogen bond prevents the receptors from exerting their normal activity, therefore advantaging its native substrate. From the above finding it can be concluded that chrysin can be used as a potent neuroprotective modulator, however further research is needed to understand the underlying mechanism of its action.

Keywords: Autism, Blood-brain barrier, Chrysin, Blood-brain barrier disrupting proteins, molecular docking.

INTRODUCTION:

Autism (ASD) is a neurodevelopmental disorder that predominantly affects an individual's personal power to engage with others and constrained stereotypic behaviour (Bhandari *et al.*,2018). The prevalence is roughly 1 in 100 children worldwide according to the World Health Organisation. Although the aetiology of Autism spectrum disorder (ASD) is unknown, it is evident that both genetic and environmental factors play a key role in the development of ASD. Since there are no biomarkers available for Autism spectrum disorder, clinical observation of patients is essential for diagnosis, which is typically made in the first three years of life (Serra *et al.,* 2019).

The blood-brain barrier (BBB) is a component of the neurovascular unit (NVU) that serves as a blood-brain interface, establishing a link between the CNS and the periphery. The BBB shields the blood from the brain, allowing serum factors and neurotoxins to be safeguarded and administered in a controllable environment. The BBB serves as a transport interface (with specific transporters), a secretory body, and a metabolic barrier in addition to acting as a physical barrier (due to the presence of specialized tight junctions and other changes that prevent unregulated leakage) (Rhea *et al.*,2019). The BBB has been implicated in a multitude of neurological disorders, notably stroke, epilepsy, multiple sclerosis, Parkinson's disease, and Alzheimer's disease (FiorentinoM *et al.*,2016). Few researches have suggested changes in BBB function in Autism spectrum disorder. Increased BBB permeability has been documented in animal models of Autism spectrum disorder which is measured with Evan's Blue (Kumar H *et al.*,2016) .

In recent years, understanding the pathology of disease has found promising new molecular therapeutic targets that are highly effective. Resistin, IL-1β, TNF-α, VCAM-1, and MMP-9 are proteins that are implicated in the structural integrity of the BBB, resulting in neurodegeneration and the onset of diverse neuropsychiatric illnesses (Mendes *et al.,* 2019). These proteins disrupt the BBB endothelium that causes BBB leakage which in turn leads to development of neuropsychiatric and neurodegenerative disorders (Nation *et al.,* 2019). A high inflammatory response by human IFNγ, IL-1β, and TNFα results in degradation of complex basement membrane structure in BBB (Bonney *et al.,* 2019) (Lv *et al.,* 2010)(Wang *et al.,* 2014). Resistin destroys microvascular endothelial cells as a consequence of increased oxidative stress and inflammation (Bonney *et al.,* 2019).VCAM1 (Vascular cell adhesion protein) serves as an attachment point for numerous immune cells and cytokines in the vicinity of the barrier, allowing them to destroy the barrier's tight junction (Elo *et al.,* 2018). MMP-9 (Matrix metallopeptidase) causes demyelination, the production of amyloid plaques, and the degradation of microvascular structural integrity, culminating in a leaking and damaged barrier.

Chrysin, a flavone, bioactive components found in a variety of fruits, vegetables, mushrooms, citrus fruits, honey, and propolis. It possesses anticancer, antioxidant, hepatoprotective, antiviral, neuroprotective, and anti-anxiety properties and inhibits neuro inflammation. Further, chrysin improves cognitive decline and has anti-amyloidogenic and neurotrophic properties (Nabavi *et al.,*2015) (Stompor-goracy *et al.,*2021). According to research, chrysin inhibits

anxiety- and depression-like behaviour via modulating the GABAA receptor. As a result, it can exert neuroprotective effects by modifying GABAergic innervation (Mishra *et al.*, 2021).

Risperidone is a Second Generation Antipsychotic (SGA) which has been approved by the Food and Drug Administration (FDA) for the treatment of irritability associated with autism since 2006. Risperidone is used to alleviate symptoms such as aggressiveness and mood swings. When risperidone is provided over a placebo, studies show a significant reduction in the symptoms linked with autism (Mano-Sousa *et al*., 2020).

The significance of chrysin in regulating BBB-pathogenic proteins and restoring BBB function is quiet unknown. Our research aimed to understand the interaction between chrysin and BBB-disrupting protein and to identify new therapeutic targets for Autism. The molecular mechanism of chrysin against BBB-disrupting proteins was determined via molecular docking.

MATERIAL & METHODS:

Data sources:

In this study the ligand chosen for the analysis were Chrysin (CID 932) and risperidone (control). The structure of chrysin and risperidone were downloaded from Pubchem database. The targets used for the study are Resistin (1RFX) (PDB Id:, IL-1β (1T4Q), TNFα (1TNF), VCAM-1 (1VSC), and MMP-9 (6ESM) and the respective structure were downloaded from Protein data bank.

Determination of physiochemical properties:

Molinspiration cheminformatics online tool (Kumar *et al.,*2017) was used to determine the compound's bioactivity score. The physiochemical properties like molecular weight, number heavy atoms, miLogP, and molar-refractivity were elucidated with the same software.

Determination of BBB permeability:

The Online BBB Predictor tool was determine the whether chrysin is BBB+ or BBB-. The BBB predictor can be accessed at http://www.cbligand.org/BBB/ (Liu *et al.*, 2014).

Determination of Pharmaco-dynamic properties:

The pharmacodynamic properties were predicted using PkCSM (Pires, Blundell, & Ascher, 2015), SwissADME (Daina *et al.,*2017), and ADMETSAR 2.0 (Yang *et al*., 2019). The parameters like GI Absorption, acute oral toxicity, AMES toxicity and skin permeation were determined. The SwissADME was used to determine if chrysin followed the Lipinski rule, Veber rule and Ghose rule.

Determination of toxic effects of ligand:

ADVERPred (Ivanov *et al.,*2018) and GUSAR database (Lagunin *et al.,*2011) were used to estimate the toxic effects of ligand.

Ligand-receptor docking using PyRx

Resisitin (1RFX), IL-1 (1T4Q), TNF- (1TNF), VCAM-1 (1VSC), and MMP-9 (6ESM) were the receptors for which the activity of the CHRYSIN ligand was studied in this work. Using the PRODRG server (Schüttelkopf & Van Aalten, 2004) and Modrefiner (Xu & Zhang, 2011), the ligand and receptors were subjected to energy minimization to meet the requirements for molecular docking analysis.

PyRx was used as the docking engine for molecular screening of all the chemical libraries (Dallakyan & Olson, 2015). The ligands were regarded as flexible during the docking process, but the protein was regarded as rigid. Using PyRx's Auto Grid engine, the configuration file for the grid settings was produced. The application was also used to identify or anticipate the amino acids that interact with the ligands in the protein's active site. Results with positional root-mean-square deviation (RMSD) less than 1.0 were regarded optimal and grouped together to achieve the best binding (Pagadala *et al.,*2017). The ligand with the greatest binding affinity was thought to have the highest binding energy (most negative).

Analysis and visualisation:

The docking data were acquired by selecting the top-scoring dock poses with high binding affinity, which were then visualized in Discovery Studio (2021 Client version) to view the ligand–receptor hydrogen interaction (Ghosh *et al.,*2021).

RESULTS & DISCUSSION:

Autism is a multifaceted illness with multiple causes that frequently co-occur. The causes of autism are unknown, although several theories including genetics, immunity, biology, and psychology that have been proposed. Although no drug has been identified so far to treat ASD, pharmacological treatments might help to reduce the symptoms including self-mutilation, aggression, repetitive and stereotypical behaviours, inattention, hyperactivity, and sleeping problems. However, complementary and alternative therapies have recently been considered in the treatment of autism.

Physiochemical properties of chrysin:

The structure of chrysin was downloaded from PubChem in SMILES and pdb format for the analysis. The physiochemical properties and bioactivity score of the ligand was scrutinized and represented in Table 1. The ligand had a Molar refractivity and TPSA (Total polar surface area) corresponding to 71.97 and 70.67 A^2 which successfully predicts the absorption of drugs(Schaftenaar & De Vlieg, 2012). The topological polar surface area (TPSA), which is the total of the surface areas occupied by the oxygen and nitrogen atoms and the hydrogens linked to them, is a physicochemical parameter that describes drug absorption, including blood-brain penetration and intestine absorption. Good intestine absorption is predicted for the novel drug candidates with TPSA values below 140 A^2, whereas good blood-brain penetration is predicted with TPSA values below A^2(Prasanna & Doerksen, 2008). According to the algorithm described by Molinspiration, the miLogP values (octanol/water partition coefficients) are less than 4, demonstrating optimal absorption and oral bioavailability. These values are derived as the total of fragment-based contributions and correction factors (Anichina *et al.,* 2021).

BBB permeability of chrysin:

The BBB permeability of the ligand was 0.018 when analysed using Online BBB Predictor and the curve obtained via. Online BBB predictor is represented in the below Figure 1. The pharmacokinetic properties, Drug Likeness and the toxic effects of the ligand investigated are presented in Table 2.

Pharmaco-dynamic of chrysin:

The pharmacodynamic analysis suggests that the ligand has high GI absorption indicating that during oral administration the drug intake is higher. The water solubility logS value is -2.777 which suggests that it is absorbed in the blood and tissues (Prakash & Gupta, 2012). The bio-availabilty score of the ligand is 0.55. The ultimate goal of a drug discovery strategy is to develop a molecule that is bioavailable in humans; rat bioavailability is usually employed as a proxy for this attribute, and compounds with low rat bioavailability may face difficulties acquiring management clearance for development (Martin, 2005). The analysis strongly confirms that the ligand obeys Lipinski rule (oral bioavailability), Ghose rule (defines the qualifying range of drug-like chemical library), Vebers rule (molecular weight cutoff), Egan rule (bioavailabilty) and Muegge rule (utilises pharmacophore filter to differentiate drug-like and nondrug-like compounds).

Toxicity of chrysin:

Toxicity investigations in animal models were carried out to validate the lethal dose of chrysin. Chrysin causes detrimental effects at doses of 844,400 mg/kg by intraperitoneal administration, 194,800 mg/kg by intravenous administration and 849,700 mg/kg by oral administration, as shown in Table 3. According to the OECD (Organisation for Economis Co-operation and Development) categorization, it is a class 4 chemical. Chrysin is BBB and CNS permeable, according to pharmacokinetic prediction; its low toxicity and mutagenic qualities indicated that it is a good candidate for the treatment of these conditions and could be effective in BBB rejuvenation.

Molecular docking of chrysin and risperidone:

The interaction of chrysin with its target receptors has been explored using PyRx to estimate the probability of the association with the target receptors with the ligand toward the active ligand-binding site Table 4 represents the interaction of chrysin with the respective targets. The nature and strength of molecular interaction, is symbolized by binding energy. The participation in hydrogen bond formation with the active site of target receptors was used to resolve the outcomes of protein–ligand interaction.

The interacting amino-acids involved in hydrogen bond interactions of chrysin and resistin are TRP and ASP. GLN and PRO are involved in the interactions between chrysin and IL-1β. GLU, GLN and SER are the aminoacids interacting between chrysin and TNF-α. VAL is involved in VCAM-1 interactions whereas ALA, LEU and GLN are involved in interactions with MMP-9. The binding energy of the ligand-receptor interactions was -5.4,-6.6,-8.6,-7.8 and -9.6 for Resistin, IL-1β, TNF-α, VCAM-1 and MMP-9 respectively. Lower binding energy represents better binding of ligand and the targets.

The binding energies of TNF-α, VCAM-1 and MMP-9 was -9.9,-9.1 and -11 representing a better binding of ligand and targets. The interacting aminoacids involved in risperidone and resistin interactions are VAL and ILE, risperidone and IL-1β are VAL and TYR, risperidone and TNF-α are GLU and GLN, risperidone and VCAM-1 are VAL and LYS and risperidone and MMP-9 are TYR,MET and GLN respectively.

The targets and their probable role in autism and BBB disruption:

The broad family of proteases known as Matrix metallopeptidase (MMP) includes many different enzymes that are vital for several critical pathologic processes, which includes inflammation, fibrosis, arthritis, and cancer. MMPs also play a key role in the normal development of the central nervous system. MMPs can regulate neuroplasticity and neurogenesis and contribute to a hyper-plasticity state associated with ASD, which makes the biological basis for their participation in the etiopathology of ASD feasible. During crucial developmental stages, their pathologic proteolytic effects on the BBB may also expose the Central nervous system (CNS) to systemic circulation (Abdallah & Michel, 2013). The blood-brain barrier is believed to leak as a result of elevated levels of MMP protein production and activity, possibly as a result of basement membrane and tight junction protein breakdown (Rempe *et al.,*2016).

Communication between cells of the innate and adaptive immune systems is facilitated by cytokines, which are cell-signalling molecules. Neurotransmitter activity, neuroendocrine activity, neurogenesis, and changes to brain circuitry are among the few of the strategies by which cytokines may affect the behavioural effect (Capuron & Miller, 2011). The harmful process of excitotoxicity may result from cytokines' increased release and decreased reuptake of the excitatory neurotransmitter glutamate (Tilleux & Hermans, 2007). An elevated excitation/inhibition ratio is important in the brain systems, including sensory, mnemonic, social, and emotional systems, which might be the basis for a model for some types of ASD supported

mechanism (Rubenstein & Merzenich, 2003). Autism-related IL-1β dysregulation can have a range of neurological effects. The balance between excitatory and inhibitory signals can be altered by changing these proteins, which might elucidate the neurological characteristics of autism (Rubenstein & Merzenich, 2003).

Peptides or polypeptides called adipokines (resistin) are made by white adipose tissue and are crucial for both normal physiology and the metabolic syndrome. Adipokines that are released into the bloodstream can interact with the BBB and have a significant impact on the CNS. Adipokines can alter endothelial function and signalling, modulate signals from other adipokines and cytokines, or permeate the BBB on their own. These are the three main ways that adipokines interact with the BBB(Pan & Kastin, 2007). Children with ASD have greater levels of resistin, which could trigger penetration of the BBB (Ghaffari *et al.,* 2016).

Children with autism exhibit higher TNFα- concentrations, suggesting that impaired innate immunity is a significant pathogenic component in autism (Ghaffari *et al.,* 2016). Under inflammatory conditions, human brain microvascular endothelial cells that compose the blood–brain barrier (BBB) release soluble vascular cell adhesion molecule-1 (sVCAM-1). VCAM-1 inhibits BBB function directly by triggering intracellular signalling events via integrin-4 (Haarmann *et al.,* 2015).

Risperidone has lowest binding energy with all the targets when compared to that of chrysin. Chrysin represents a binding energy closer to that of risperidone stating that it can bind similar to that of risperidone. The interactions with chrysin by establishing robust hydrogen bonds may attribute to the hindrance of targets from exerting their normal activity. The chrysin and receptor interactions are depicted in Figure 2 followed by risperidone and targets in Figure 3. The 2-dimensional presentation represents the type of bonds interacting and the active site involved in hydrogen bond formation Figure 4 represents chrysin and its target Figure 5 represents risperidone and its targets.

The results obtained from the *in silico* analysis strongly advocates the prospective action of chrysin against BBB disrupting proteins

CONCLUSION:

To conclude, from the results of the current investigation chrysin has potential neuroprotective ability to act against BBB disrupting proteins. Chrysin might be the next promising single-drug candidate for treating and reviving neurological abnormalities, which has an effective pharmaco-dynamic profile. The development of rodent or human BBB models to investigate the impact of these proteins is needed to support the current findings. As a result, the scientific community will be able to examine the efficacy of chrysin in these models and assess whether it might be exploited as a tool to comprehend drug dynamics and their pharmacological influence on BBB regeneration before clinical trials. Chrysin has multifaceted activity against neurological disorders, suggesting chrysin as a probable candidate for further *in vitro* and *in vivo* research.

Table 1: Physiochemical properties of chrysin

Formula	$C_{15}H_{10}O_4$
Molecular weight	254.24g/mol
Number of heavy atoms	19
Number of aromatic heavy atoms	16
Number of rotatable bonds	1
Number of H-bond acceptors	4
Number of H-bond donors	2
Molar Refractivity	71.97
TPSA	$70.67A^2$
miLogP	2.94
nON	4
nOHNH	2

Table 2: Pharmacodynamic properties of chrysin

Parameter	**CHRYSIN**
GI Absorption	High
BBB PERMEANT	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (Skin permeation)	-5.35cm/s
Lipinski rule	Yes; 0 violations

Veber rule	Yes
Ghose rule	Yes
Egan rule	Yes
Muegge rule	Yes
Bioavailability score	Yes
$CaCo_2$ permeability	0.945 Numeric (log Papp in 10^{-6} cm/s)
Intestinal absorption (human)	93.761%
CNS permeability	-1.912 Numeric (log PS)
Total Clearance	0.405 Numeric (logml/min/kg)
AMES toxicity	No
hERG I inhibitor	No
hERG II inhibitor	No
Hepatotoxicity	No
Acute Oral toxicity	1.775 Kg/mol

Table 3: Rat acute toxicity predicted by GUSAR

COMPOUND	ROUTE OF ADMINISTRATION	Log 10(mmol/Kg)	mg/Kg	LD50 OECD Chemical classification
CHRYSIN	Intraperitoneal	0,521	844,400	Class 5
	Intravenous	-0,116	194,800	Class 4
	Oral	0,524	849,700	Class 4

Table 4: Interactions of chrysin and risperidone with specific targets

RECEPTOR	INTERACTING AMINOACIDS	BINDING ENERGY (Kcal/mol)	HYDROGEN BOND LENGTH	TYPES OF BOND
CHRYSIN				
Resisitin (1RFX)	TRP,ASP	-5.4	5.82,3.51	Vanderwaals, conventional hydrogen bonds,Pi-Anion, Pi-Pi T-shaped, Pi-Alkyl
IL-1β	GLN,PRO	-6.6	4.91,5.61	Vanderwaals, conventional hydrogen bonds,Pi-Cation, Pi-Pi stacked , Pi-Alkyl
TNF-α (1TNF)	GLU,GLN,SER	-8.6	5.22,5.19,2.95	Vanderwaals, conventional hydrogen bonds,Pi-Anion
VCAM-1 (1VSC)	VAL	-7.8	8.94	Vanderwaals, conventional hydrogen bonds, carbon hydrogen bonds,Pi sigma and Pi-Alkyl
MMP-9 (6ESM)	ALA,LEU,GLN	-9.6	2.54,1.80,1.99	Conventional hydrogen bonds,Pi-Sigma, Pi-Pi Stacked, Pi-Pi T-shaped, Pi Alkyl
RISPERIDONE				
Resisitin (1RFX)	VAL,ILE	-6.9	3.47,4.31	Vanderwaals, conventional hydrogen bond, carbon hydrogen bond, halogen, Pi-Anion, Pi-Donor hydrogen bond, Pi-Pi stacked, Alkyl, Pi-Alkyl
IL-1β	VAL,TYR	-7.5	4.31,5.44	Vanderwaals, conventional hydrogen bond, carbon hydrogen bond, Alkyl, Pi-Alkyl
TNF-α (1TNF)	GLU,GLN	-9.9	5.71,5.57	Vanderwaals, conventional hydrogen bond, carbon hydrogen bond, Pi-Anion, Pi-Alkyl
VCAM-1 (1VSC)	VAL,LYS	-9.1	3.91,5.30	Vanderwaals, conventional hydrogen bond, carbon hydrogen bond, halogen, Pi-Donor hydrogen bond, Pi-Pi stacked, Amide-Pi stacked, Alkyl, Pi-Alkyl
MMP-9 (6ESM)	TYR,MET,GLN	-11	4.93,5.40,5.01	Vanderwaals, conventional hydrogen bond, Pi stacked, Alkyl, Pi-Alkyl

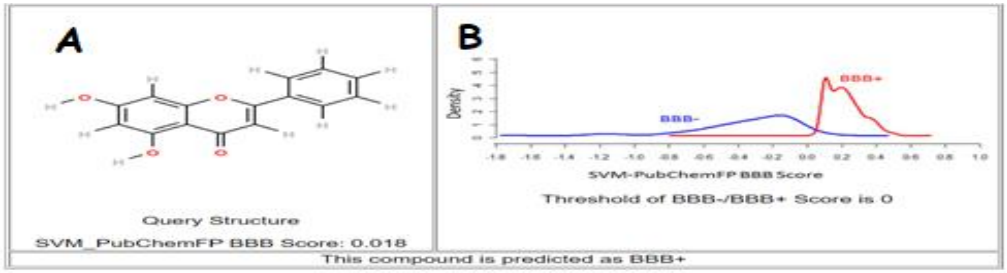


Figure 1: A. BBB Permeability score of chrysin B. Graphical representation of BBB permeability

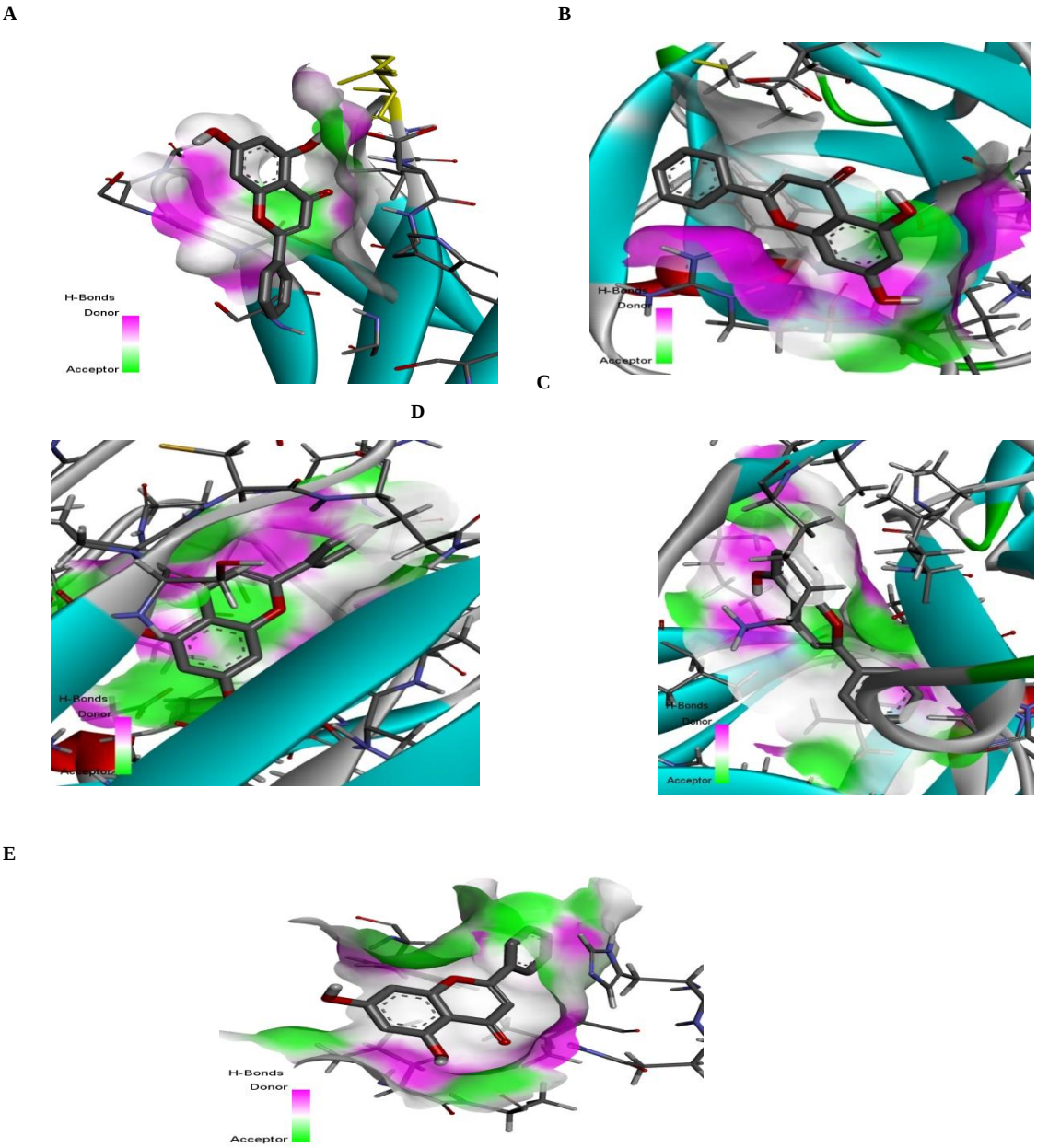
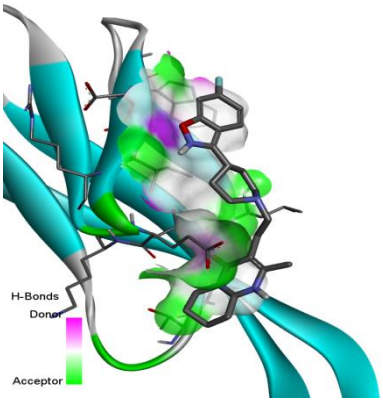


Figure 2: Molecular representation of chrysin and target interactions. A-Resistin, B-IL-1β, C- TNF-α, D-VCAM-1, E- MMP-9

A



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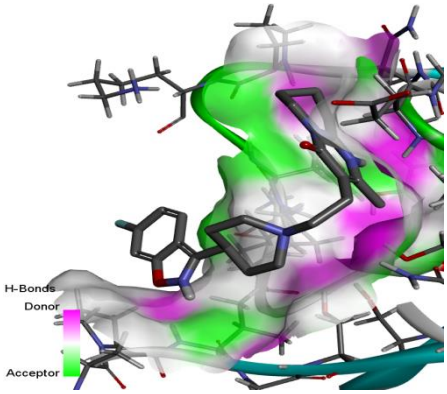

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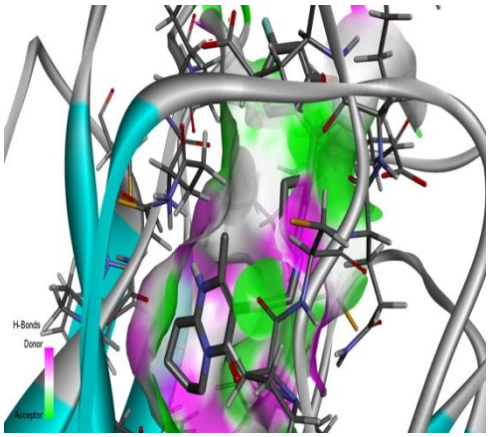

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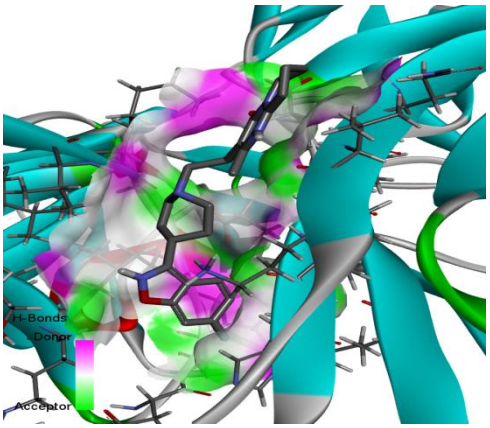

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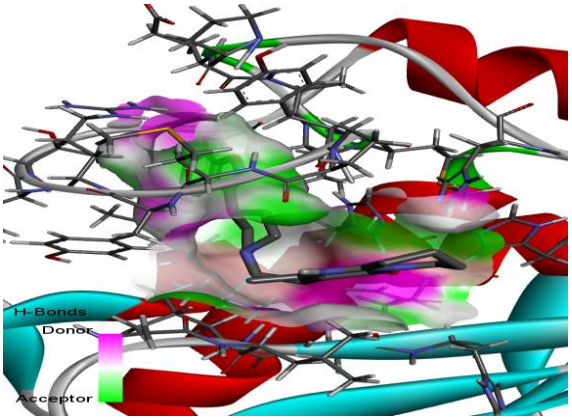

Figure 3: Molecular representation of risperidone and target interactions. A-Resistin, B-IL-1β, C- TNF-*α*, D-VCAM-1,E- MMP-9

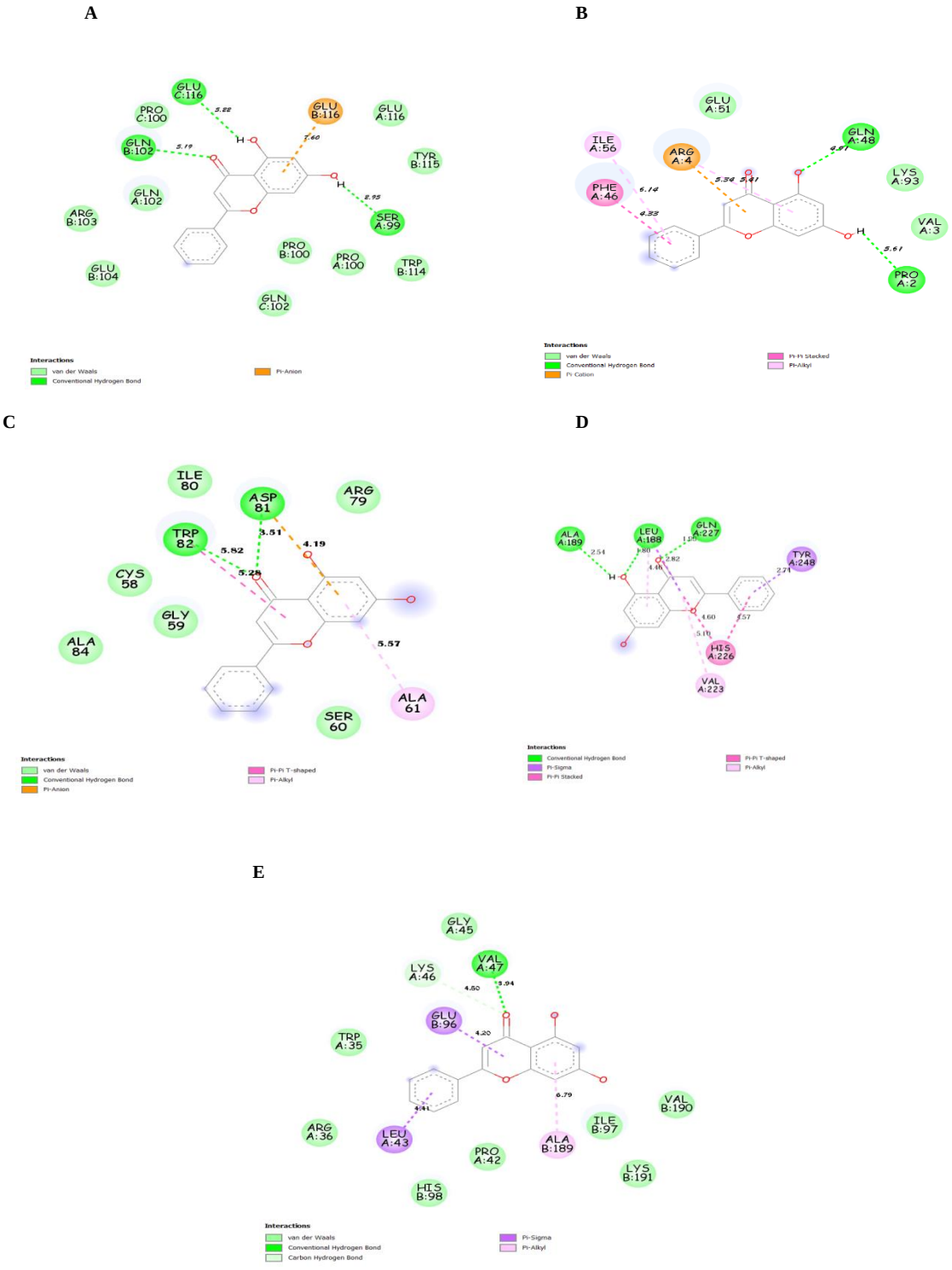

Figure 4: 2D-Representation of interacting aminoacids – chrysin. A-Resistin, B-IL-1β, C- TNF-α, D-VCAM-1, E- MMP-9

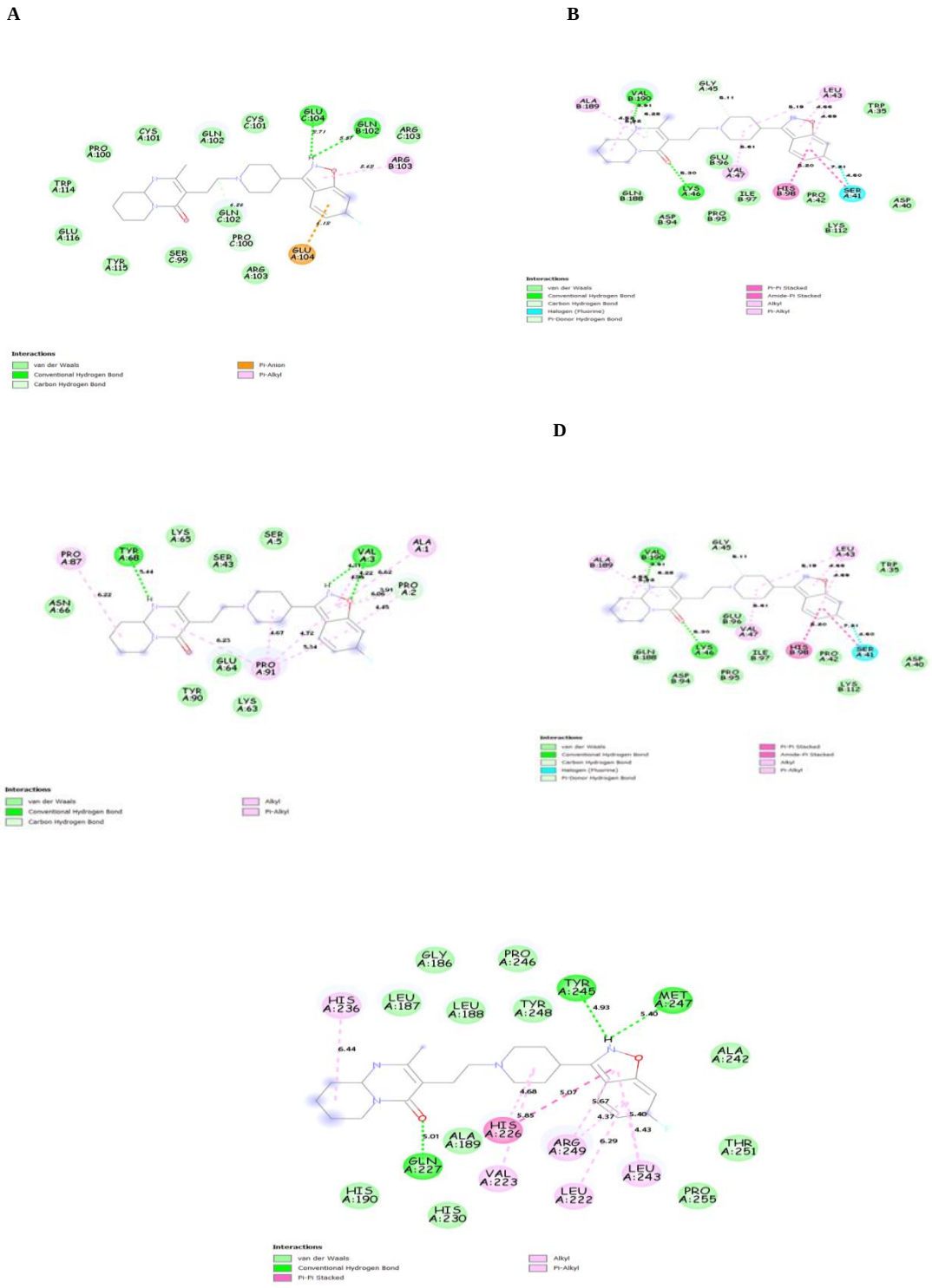

Figure 5: 2D-Representation of interacting aminoacids –Risperidone. A-Resistin, B-IL-1β, C- TNF-*α*, D-VCAM-1, E- MMP-9

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