Exploring the Inhibitory Potential of Thiazolidinedione based drugs against HN, F, M and N Proteins of NDV through Computational Analysis

Samra Komal

Reg No. 00000327462

Master of Science in Industrial Biotechnology

Supervisor

Dr. Najam us Sahar Sadaf Zaidi

Department of Industrial Biotechnology

Atta-Ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

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A thesis submitted in partial fulfilment of the requirement for the degree of

Master of Science in Industrial Biotechnology

By

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National University of Sciences & Technology

MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by:
(Student Name & Regn No.) Samra Komal Reg No. 00000327462

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Examination Committee Members

1. Name: Dr. Saadia Andleeb   Signature: _____

2. Name: Dr. Muhammad Tahir   Signature: _____

3. Name: Dr. Farhan Afzal   Signature: _____

Supervisor's name: Dr. Najam us Sahar   Signature: _____ 27/9/23

Sadaf Zaidi

Professor
NAJAM US SAHAR SADAF ZAIDI, PHD
Dept. Industrial Biotechnology, ASAB
National University of Sciences and
Technology (NUST), Islamabad

Dr. Amjad Ali, PhD
Head of Department (HoD)
Industrial Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

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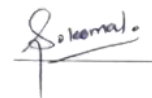Head of Department   Date   Date

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_____
Dean/Principal

Prof. Dr. Muhammad Asghar
Principal
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST Islamabad

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I, Samra Komal, declare that this research work entitled "**Exploring the Inhibitory Potential of Thiazolidinedione based drugs against HN, F, M and N Proteins of NDV through Computational Analysis**" is my own work. The work has not been presented elsewhere for assessment. The work here in was carried out while I was a post-graduate student at Atta-ur-Rahman School of Applied Biosciences, NUST under the supervision of Dr. Najam-us -Sahar Sadaf Zaidi. The material that has been used from other sources has been properly acknowledged / referred.

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Reg No: 00000327462

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Signature:

Supervisor: Dr. Najam Us Sahar Sadaf Zaidi

Date: 6/10/23

Signature (HOD):

Dr. Amjad Ali

Date: 9/10/23

Signature (Dean/Principal):

Dr. Muhammad Asghar

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Dr. Najam Us Sahar Sadaf Zaidi

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Dedicated To

My Father

Sheraz Hussain

For all the sacrifices he made…

Especially,

My Nano

Nighat Yasmeen

For believing in me always…

My Dearest Khala, and all my Mamoo…

For their support throughout this journey

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List of Abbreviations

AAvV   Avian Avulavirus

APMV   Avian Paramyxovirus

F   Fusion Protein

HN   Haemagglutinin-Neuraminidase

Kda   Kilodaltons

L   Large protein

M   Matrix Protein

NDV   Newcastle Disease Virus

ND   Newcastle Disease

NP   Nucleocapsid Protein

Nt   Nucleotides

OIE   World Organization for Animal Health

P   Phosphoprotein

R&D   Research and development

ABSTRACT

The Newcastle Disease Virus (NDV), primary pathogen responsible for Newcastle Disease (ND) infection in poultry, is classified as an avian paramyxovirus type I and possesses an outer protective layer. The viral genome consists of a single-stranded RNA molecule with a negative-sense orientation, measuring around 15.2 kilobases in length. This RNA molecule encodes a total of six distinct proteins. Four structural proteins including HN, F, M and NP are regarded as promising therapeutic targets owing to their crucial role in viral replication cycle. There are currently no NDV specific antiviral agents/therapeutics available in the poultry industry and due to the ineffectiveness of commercial live attenuated vaccine, outbreaks of this disease are frequently observed within the chicken business, resulting in substantial economic repercussions. The Thiazolidinedione based drugs known for their anti-diabetic, anti-microbial, anti-tumor, anti-viral, anti-inflammatory, and anti-cancer properties have been repurposed as anti-NDV drugs through computational methods. Nevertheless, there has not been a single study about these Thiazolidinedione based drugs reported against NDV. Therefore, the present study has been conducted to explore the inhibitory potential of 10 Thiazolidinedione based drugs against four essential structural proteins of Newcastle Disease Virus i.e., HN, F, M and NP. Significant and encouraging outcomes have been shown for the following compounds: HN/Troglitazone (-9.2 kcal/mol), HN/Englitazone (-9.1 kcal/mol), NP/Troglitazone (-8.6 kcal/mol), and F/Balaglitazone (-7.8 kcal/mol). In a similar vein, the compounds M/Rosiglitazone and M/Ciglitazone have demonstrated potential positive results, with respective binding energies of -8.4 kcal/mol and -8.1 kcal/mol. The structural flexibility of the HN/Troglitazone, HN/Englitazone, NP/Troglitazone, and F/Balaglitazone docking complexes was confirmed by MD simulations, as evidenced by the root mean square fluctuation (RMSF) values below 5Å. The study's findings indicated that Troglitazone, Englitazone, Balaglitazone, Rosiglitazone, and Ciglitazone have the most

effective antiviral therapeutic potential against Newcastle Disease infections. Additional in vitro and in vivo evaluation to better understand the molecular complexity of therapeutic and antiviral capabilities will increase researchers' chances of finding innovative therapeutics throughout the drug development process.

INTRODUCTION

The Newcastle Disease Virus (NDV) is an enveloped virus that is responsible for causing Newcastle Disease. It possesses a genome consisting of single-stranded negative-sense RNA. The virus in question belongs to the Avulavirus genus of the Paramyxoviridae family. (Ganar et al., 2014). The virus is typically limited and mildly zoonotic in nature and causes a highly acute and extremely contagious respiratory illness in birds called Newcastle disease. The virus was initially identified in the year 1926 at a place called Newcastle, United Kingdom and since then it has been spreading worldwide devastating poultry industries and costing the economy billions of dollars (Phale, 2018a). The NDV virus is not just a threat to domestic chickens, but it has also been causing rare illnesses in other wild bird populations as well. Initially, the Newcastle disease virus was taxonomically named Avian paramyxovirus 1 (APMV-1), however, the name has been recently changed to Avian Avulavirus 1 (AAvV-1). The virus that causes Newcastle disease, known as AAvV-1, is one of the 19 members of the genus Avulavirus. The genus Avulavirus is classified within the family Paramyxoviridae, which belongs to the order Mononegavirales. (Amarasinghe et al., 2017).

The virus is pleomorphic, with a single strand of RNA genome consisting of 15,186 bases of nucleotides and helical capsid symmetry. Furthermore, it also has six transcriptional units that encode the three envelope proteins and three core proteins listed below. One of the envelope proteins exhibits the activities of both haemagglutinin and neuraminidase (HN), the second one exhibits the fusion activity (F) and the third one matrix (M) is located inside the envelope. The core proteins are nucleocapsid protein (NP), the large protein (L) and phosphoprotein (P) (Czeglédi et al., 2006). HN and F protein are immunogenic proteins in nature, also the most significant proteins in determining the virulence and infectivity of the virus because of their role in activating membrane fusion and viral entry in host cells. The sequence of the above mentioned six proteins is as; 3'- NP- P-M- F- HN- L- 5' (Phale,

2018b). NDV, like other paramyxoviridae, produces the proteins V and W as a result of the translation of the alternative mRNA for the P protein. During transcription of the P gene, RNA editing generates alternative mRNAs (P. L. Rao et al., 2020).

The NDV has been categorized into two more classifications, namely Class I and Class II. Class II consists of a total of 16 genotypes. Based on the findings of Diel et al. (2012), it has been established that Class II genotypes III–IX and XI–XVI exhibit a significant degree of pathogenicity, as supported by many sources. Various strains exhibit varying affinities for different organs, and the degree of pathogenicity also differs among these strains. The categorization of NDV into distinct pathotypes is determined by assessing the nature and severity of symptoms induced in hens. There are three pathogenic strains that have been categorized as follows: the lentogenic strain, which is characterized by low virulence; the mesogenic strain, which exhibits intermediate virulence; and the velogenic strain, which is very virulent. According to Alexander (1988), the velogenic strain can be divided into two distinct categories: the viscerotropic-velogenic strain, which is characterized by its ability to cause deadly hemorrhaging, and the neurotropic-velogenic strain, which is associated with the development of neurological diseases.

The classification of the virulence of Newcastle Disease Virus (NDV) strains is based on the presence of specific types of basic amino acids inside the proteolytic fusion cleavage site of the F protein. This factor possesses the capacity to elucidate the molecular underpinnings of variability in virulence. The process of fusion, along with the contacts between cells and between cells and viruses, is facilitated by the presence of basic amino acids near the site of fusion. The majority of highly pathogenic variants of Newcastle Disease Virus (NDV) exhibit the amino acid phenylalanine (F) at position 117, along with three or more residues of either lysine (K) or arginine (R) at position 113. (Brown & Bevins, 2017a).

Enveloped viruses employ diverse fusion methods in order to invade their host cells. There are two primary mechanisms by which viral fusion with host cells can occur: (i) Direct fusion, where the envelope membrane of the virus directly merges with the cell membrane of the host, and (ii) receptor-mediated endocytosis, where a receptor is involved in facilitating the fusion process and nucleocapsid is translocated inside the host cell as a result of endocytosis (D. S. Dimitrov, 2004). When a virus enters the host body, then the proteases present inside the host are responsible for cleaving the precursor F0 Fusion protein into F1 and F2 proteins. This cleavage will set in motion multiple hemolytic mechanisms, including infection by fusion. The viruses which contain an active cleavage site are virulent because any type of protease, present inside the host body, can cleave the F protein, and start the infection. Whereas, in the absence of an active cleavage site, only trypsin and trypsin mediated enzymes can cause the cleavage of the fusion proteins. Due to the prevalence of these enzymes in the respiratory and gastrointestinal tracts, replication on the host may be limited (Huang et al., 2004) (Nagai & Klenk, 1977)

NDV is the infectious agent behind a devastating chicken disease called Newcastle Disease. This virus has been found in more than 250 different bird species, making it one of the deadliest diseases in the poultry business. Based on the strains responsible for causing the infection, NDV can be divided into three forms: (a) mild or lentogenic form (b) moderate or mesogenic form (c) virulent or velogenic form. The highly virulent form of Newcastle disease is also called Exotic Newcastle Disease (END). END is regarded as the most virulent disease of poultry. It affects all species of birds. This disease is so virulent that most of the birds die without displaying any clinical symptoms. Luckily, END has no known human health effects (Wakamatsu et al., 2006). The inclusion of the highly pathogenic variant of Newcastle disease in the Terrestrial Animal Health Code of the World Organization for Animal Health (OIE) necessitates its mandatory reporting to the OIE (OIE 2004).

The most common mode of transmission of NDV is through direct contact with carrier or diseased birds. The virus is shed into their feces by infected birds thus contaminating the area surrounding them. Then the transmission can easily occur by aerosol exposure or by coming direct in-contact with either the infected bird itself or with the oral and fecal discharge, contaminated food, equipment, water and even by clothing. It can sustain for many weeks in the environment especially in winter (Estola et al., 1979).

The signs and symptoms of the infection vary based on the type of NDV strain causing the infection. It can also depend upon the species, health, and age of the host bird. Once the virus has infected a host, the incubation period might last anywhere from four to six days. Young birds as young as 2 days old (on average 5) can start showing symptoms. The spread by aerosol exposure is faster as compared to the spread by fecal-oral route. Sneezing, coughing, rales, and gasping are the most common respiratory symptoms of NDV infection. Complete paralysis, circling, tremors, clonic spasms, and paralysis of the legs and wings are all symptoms of nervous system dysfunction which can also be seen (Absalón et al., 2019). Most cases of nervous behavior in birds are found in captive exotics and cormorants. Pigeons frequently exhibit symptoms of diarrhea and nervous system disorders. The laying of eggs might cease, either temporarily or permanently. Eggs may have an unusual texture, appearance, or size, and their albumen may be watery and see-through (Roberts et al., 2011). In well vaccinated birds, the signs and symptoms are not properly visible except for the decline in egg production. However, they can still shed virus in saliva and feces and can easily spread infection to poorly vaccinated birds, which may be able to develop torticollis, ataxia and other symptoms 2-14 days after the exposure and can recover with supportive care. Gross lesions are visible in the event of viscerotropic velogenic strain. Most commonly seen histopathological abnormalities include pneumonia, tracheitis, myocarditis, pericarditis,

atropy of Bursa Fabricious, nephritis interstitial, encephalitis, and splenitis (Etriwati et al., 2017).

Newcastle disease has a high mortality rate in domestic birds and is responsible for the devastating economic losses in poultry industry (Lancaster, 1976). Since most of the birds are not routinely vaccinated, the disease can easily spread with direct contact between diseased and healthy birds thus resulting in mass mortality of poultry chickens. Newcastle disease is still regarded a severe threat to the chicken industry and, by extension, the economy, despite significant advances in treatment, diagnosis, and vaccination since the 1950s (Phale, 2018a). In 2002-03, the United States had a loss of US$162 million due to a severe epidemic of Newcastle disease in California. Four million birds died as a direct result of this outbreak (Cattoli et al., 2011). In Pakistan, the poultry industry is the second largest industry after textile industry. The poultry sector is one of the most important zones of agriculture industry in Pakistan with a massive contribution of 1.3% in national GDP (Rehan et al., 2019). The recent reported ND outbreak in Pakistan during 2012 caused a loss of 6 billion Pakistani Rupees. Since the 1960s, the poultry industry has been commercially providing a significant contribution of 26.8 % meat production and 5.76% of eggs to the population (Hussain et al., 2015). Poultry sector in Pakistan saw 20-30% growth per anum in early 1970s and about 10-15% in 1980s making it one of the most important sectors contributing to economy with a consumption of almost 4% per anum. The poultry industry is also an employment source for almost more than 1.5 million people (Usman, 2016).

Since, there are no specific effective antiviral agents/therapeutics available against NDV and despite the availability and extensive use of live attenuated vaccine against NDV, significant outbreaks have been observed all over the world because of genetic and antigenic variations, resulted from continuous evolution.

Research into antivirals, which can halt the transmission of viruses or halt disease, is underway alongside the development of various vaccine candidates but, significant time, money, and effort are needed to create a novel therapeutic product. From target identification to marketing approval, novel drugs typically take over 12 years. This is consistent with analyses conducted across all therapeutic domains. The average cost to produce a New Biological Entity is projected to be around $2.6 billion. This is significantly more than the cost to develop a New Molecular Entity (Mohs & Greig, 2017). Before a new drug may be used on the public, it must first pass rigorous testing and be manufactured to exacting standards. Research and development (R&D) for novel chemical entities is more expensive due to all these factors (Dickson & Gagnon, 2009).

Drug Repurposing means finding new therapeutic uses for existing drugs. These drugs may be modified versions of currently available pharmaceuticals, compounds that have been approved but never sold, compounds that have been used before but now restricted due to some other complications caused by them, or compounds that were never used in clinical trials. Drug repurposing, in a nutshell, is the process of finding new indications for existing drugs. Since the risk of clinical failure is smaller and the investment needed to reach the market is greatly lowered, pharmaceutical corporations are also incentivized to invest in such drug repurposing programs. Hence, the current investigation was designed to examine the inhibitory capacity of repurposed Thiazolidinediones against Newcastle Disease Virus (NDV). Thiazolidinediones are key components of many commercially available drugs as anticancer, antimicrobial, antitumor, antidiabetic, anti-inflammatory, antifungal, antiviral, anti-HIV, cytotoxicity and anti-hypernociceptive compounds (Sahiba et al., 2020). This research set out to identify whether these 10 Thiazolidinediones (Pioglitazone, Englitazone, Troglitazone, Balaglitazone, Ralitoline, Etozoline, Lobeglitazone, Rosiglitazone, Ciglitazone and Teneligliptin) had any effect on blocking the activity of key NDV structural proteins (F,

HN, M, NC). Multiple pharmacokinetic analyses were conducted to evaluate the drug-likeness and toxicity potential of Thiazolidinediones as potential leads for the development of an anti-NDV drug. Additionally, molecular docking and MD simulation studies were carried out to assess the stability of the docked structures formed by the ligand-protein complexes.

1.1. Aim & Objectives

The proposed research aims to investigate the feasibility of repurposing anti-diabetic therapeutics as anti-viral drugs for use in the poultry sector.

The objective of this study includes:

- To explore inhibitory potential of Thiazolidinedione based drugs against Newcastle Disease Virus through computational analysis.
- Evaluation of pharmacokinetic properties, bioactivity score, toxicity potential and validation through MD Simulation.

LITERATURE REVIEW

2.1. Newcastle Disease Virus

Newcastle disease (ND) is a highly fatal disease that poses a significant threat to poultry, with the potential to cause severe economic consequences for the global domestic poultry sector. The virus accountable for inducing this fatal disease is the Newcastle disease virus, initially discovered in Newcastle, England, and afterwards in Indonesia in the year 1926. Based on the most recent unified phylogenetic classification scheme and new nomenclature for Newcastle disease virus, it has been recently designated as Avian orthoavulavirus 1 under the Orthoavulavirus genus, Avulavirinae subfamily, and Paramyxoviridae family (Zhang et al., 2023).

The NDV, also known as Newcastle Disease Virus, is a zoonotic virus that may be classified into several pathotypes based on the mean death time (MDT) seen in chicken embryos. Lentogenic strains, which are characterized by their non-virulent nature, have a minimum duration of more than 90 hours for the median death time (MDT). The mesogenic bacteria, which exhibit moderate virulence, demonstrate a mean death time (MDT) ranging from 60 to 90 hours. In contrast, the velogenic strains, characterized by high virulence, exhibit an MDT of fewer than 60 hours. The United States experienced a highly damaging outbreak of velogenic Newcastle Disease Virus (NDV) during the period of 2002-2003, which necessitated the euthanization of around 3.16 million avian specimens. This extensive culling operation incurred a financial burden of over $121 million. Strains of NDV exhibiting diminished virulence have been observed in avian populations, encompassing both domestic and wild bird species across various geographical regions globally (Brown & Bevins, 2017b).

During the last significant pandemic of ND in the United States, approximately 4 million birds perished, resulting in a loss of approximately $160 million. Recent NDV epidemics in

commercial poultry in Pakistan in 2012 caused enormous economic losses. NDV has killed approximately 45 million poultry animals in Punjab alone, resulting in a loss of six billion Pakistani rupees (PKR). 190 peacocks also perished in Lahore's Jallo Wildlife Park as a result of 2012 NDV outbreak (Rehan et al., 2019).

A total of 241 avian species have been documented as hosts of the Newcastle Disease Virus (NDV). The existence of Newcastle disease virus (NDV) within natural bird populations poses challenges in disease management and potentially contributes to its dissemination on a national and global scale. (Thomas & Walmsley, 2018).

2.2. Classification

The NDV isolates are categorized as members of the Avulavirus genus, which belongs to the Paramyxoviridae family. At present, a total of 16 serotypes of avian paramyxovirus (APMV-1 to APMV-15) have been discovered in diverse avian populations, encompassing both wild and domesticated species. These viruses have been associated with respiratory disorders and a notable reduction in egg output (Bello et al., 2018). All strains of NDV correspond to a singular serotype of Avulavirus; nevertheless, these strains can be categorized into two distinct classes, namely Class I and Class II. Class I strains are commonly obtained from wild bird populations and exhibit a relatively low level of pathogenicity. In contrast, class II strains are frequently obtained from domesticated poultry and can vary in their level of pathogenicity, ranging from low to high.

2.3. Molecular Biology of NDV

The genome of Newcastle Disease Virus (NDV) consists of a single-stranded, antisense RNA molecule that is responsible for encoding a total of eight gene products. These gene products include the nucleocapsid protein (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), RNA polymerase (L), as well as the V and W proteins.

The proteins are arranged in the following order: 3'- NP- P-M- F- HN- L- 5' as depicted in Figure 2.1. The genome size of NDV is reported to be either 15,186, 15,192, or 15,198 nucleotides (nt) (Zhang et al., 2023).
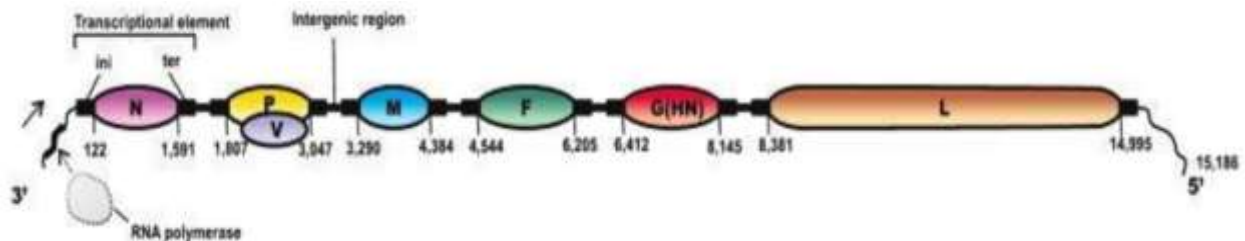
Figure 2.1: Schematic diagram of NDV genome encoding eight proteins; P (Phosphoprotein), NP (Nucleocapsid protein), F (Fusion protein), Matrix protein (M), Heamagglutinin-Neuraminidase (HN), Large protein (L) and V protein (V) (Lvov et al., 2015).

The various components of the virus work together in a cooperative manner to successfully execute the entirety of the invasion and infection process. Proteins possess unique functionalities and operate synergistically with one other. In terms of morphology, the size of virions in Newcastle Disease Virus (NDV) exhibits variation within the range of 150-300nm. These virions display characteristics of enveloped particles, featuring projections of F and HN spike glycoproteins. These glycoproteins play a crucial role in initiating the infectious cycle of the virus. The M protein is located directly below the viral envelope and plays a crucial role in facilitating the processes of viral packaging and budding. The remaining three proteins have been identified to carry out actions related to replication and are closely linked to the viral genome. The stable encapsulation of the Newcastle Disease Virus (NDV) genome is primarily attributed to the NP protein, with auxiliary assistance from the P and L proteins. The P protein plays a crucial role in the process of viral RNA production and also aids in maintaining the solubility of the NP protein. The L protein, being the biggest protein inside

the viral structure, is believed to possess a variety of activities. It plays a role in cellular

processes such as genome replication, transcription, and regulation of viral replication. The

nonstructural proteins, namely the W and V proteins, are generated through the RNA editing

process of the P gene. The V protein has a crucial role in suppressing the secretion of host

type I interferon (IFN) and inducing apoptosis, hence facilitating viral multiplication. The W

protein is an additional RNA editing product derived from the P gene. Its expression location,

whether in the nucleus or cytoplasm, is contingent upon the genotype of the viral strain (Bello

et al., 2018).
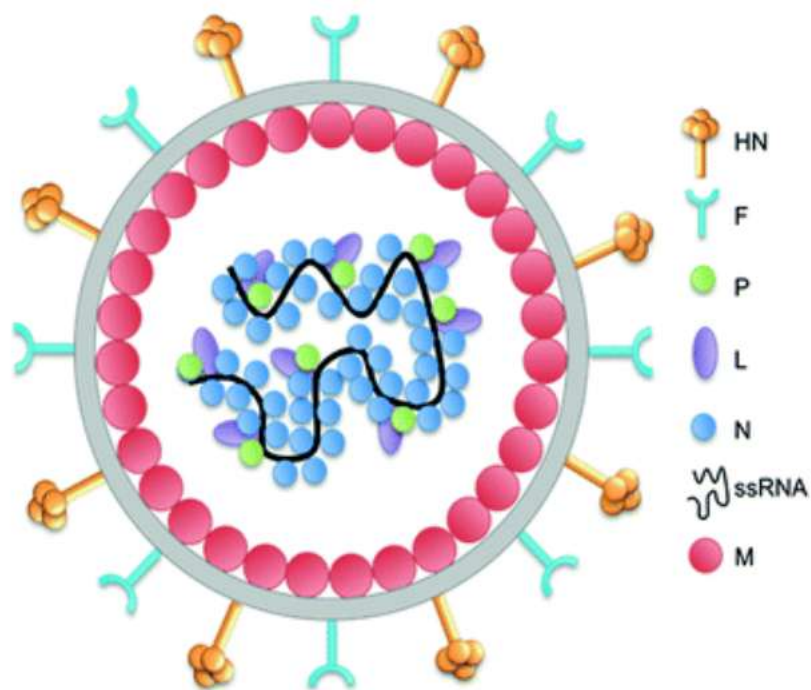
Figure 2.2: The structure of Newcastle disease virus (Thomas & Walmsley, 2018).

2.4. Target Viral Proteins

2.4.1. Fusion Protein

The fusion protein (F protein) is a glycoprotein located on the envelope of Newcastle disease

virus (NDV). It spans approximately 1792 nucleotides and encodes a lengthy polypeptide

chain consisting of 553 amino acids. The primary functions of the F protein include facilitating virus entry, promoting cell fusion, and inducing hemolysis. Initially, it is synthesized as a precursor protein known as F0. Following cleavage into F1 and F2 polypeptides, it becomes capable of mediating fusion between the virus and host cell membranes. The cleavage process is executed by proteases present in the host cell, and the susceptibility of different strains of F proteins to specific proteases is determined by the presence of distinct amino acid motifs at the cleavage site. Consequently, the cleavage by host cell proteolytic enzymes results in the formation of F1 and F2 subunits, which are joined together by a disulfide bridge, as depicted in the accompanying figure 2.3 (Dutch, 2010). Regarding the pathogenicity of NDV strains, the molecular composition of the cleavage site (known as the F protein cleavage site or FCS) holds the greatest influence.
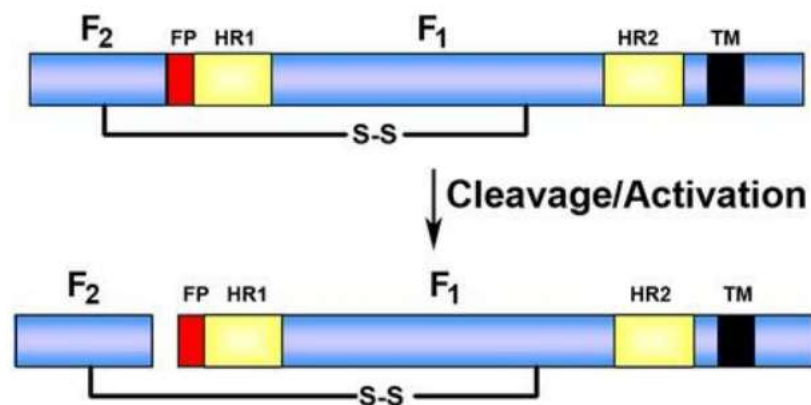
Figure 2.3: Schematic diagram of F protein activation. Inactive precursor F0 is cleaved into F1 and F2 subunits by host proteolytic enzymes (Dutch, 2010).

2.4.2. Haemagglutinin-Neuraminidase Protein

The HN glycoprotein of Newcastle Disease Virus (NDV) is an essential antigenic determinant characterized by a molecular weight of 74 kilodaltons (kDa) and a nucleotide length of around 1998, that encodes for a long polypeptide consisting of 577 amino acids

(Phale, 2018a). The HN can easily bind with sialic acid, so it enables the virus to bind with those receptors containing sialic acid. After binding to the sialic acid containing receptor, it mediates the neuraminidase activity i.e., enzymatic cleavage of the sialic acid. Along with these activities, it also aids in fusion activity by interacting it with F protein.

Previous research suggests that HN exists as a homo-tetramer comprised of disulfide-linked dimers within cells that are infected by the virus. It serves as a multifunctional protein, playing a crucial role in receptor recognition, specific membrane fusion, infection, and the pathogenesis of Newcastle disease virus (NDV) (George & Heringa, 2002).

2.4.3. Matrix Protein

Matrix protein is the third NDV envelope soluble protein that coats the inside surface of the viral envelope, has a molecular weight of 40 kDa and a length of 364 amino acids, is believed to play a pivotal role in viral protein-protein interactions by complexing with both the viral glycoprotein and the RNP core at the viral assembly site (Worku & Teshome, 2020). During viral assembly, M protein associates with N protein with the help of its net positive charge. The M protein, which sits between the nucleocapsid and the lipid membrane, is hydrophobic and lacks membrane-spanning peptides. M protein is thought to have several roles, including regulation of viral RNA production, interaction with actin, and participation in virion assembly on the host cell membrane (Peeples & Bratt, 1984). It has been hypothesized that M protein is in charge of keeping the nucleocapsid in a round shape. M protein also interacts with the plasma membrane of the host cell, which aids the budding process of the virus. Since the M protein contains its own nuclear localization sequences, it is not dependent on other NDV proteins for this process. The matrix protein has a crucial role in orchestrating the temporal and spatial aspects of maturation and infection processes. It actively participates in the sequential events that occur during budding and fusing. The NDV M protein is a crucial

multifunctional viral protein that serves significant roles in the life cycle of the virus (Worku & Teshome, 2020).
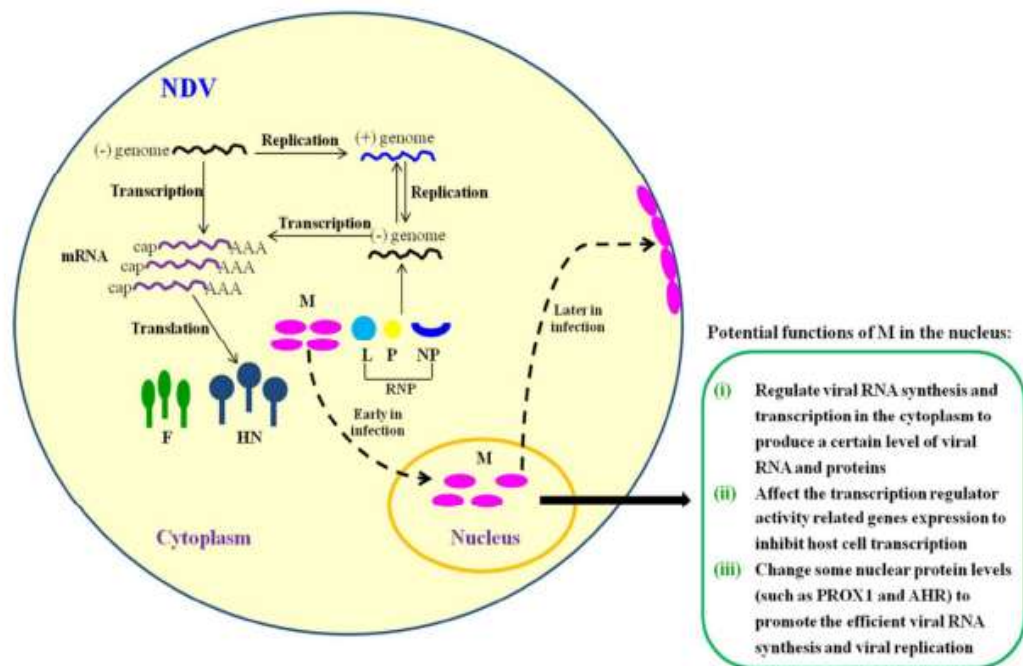
Figure 2.4: The replication and transcription of NDV genome and the potential functions of M protein in the nucleus

2.4.4. Nucleocapsid Protein

The nucleocapsid protein possesses a total of 489 amino acids (aa) and exhibits a molecular weight of 55 kDa. This protein is highly abundant within the viral particle. The electron micrograph exhibits a herringbone-like structure. The helical nucleocapsid core structure of the virus is formed in conjunction with the genomic RNA. The ribonucleoprotein complex (RNP) is formed by the genomic RNA in conjunction with the N, P, and L proteins. This complex serves as a template for RNA synthesis (Errington & Emmerson, 1997). The interaction between viral RNA and the N protein is facilitated by the amino-terminus of the protein, which also plays a crucial role in the creation of a herringbone-like structure. The formation of herringbone-like structures, which bear morphological resemblance to the

characteristic nucleocapsid structure, has been documented in the expression of recombinant NDV N protein using baculovirus.

2.5. Currently Reported Therapeutics in Experimental Phase

At present, there is a lack of commercially accessible antiviral treatments specifically targeting Newcastle Disease Virus (NDV) in the poultry industry and vaccination and antibiotics is the primary approach for controlling NDV worldwide, but many therapeutics and extracts have shown great results in experimental phase at Laboratory, and some are being tested against the virus in the market.

In 2015, Aguilar-Briseno and the team extracted sulphated polysaccharides from Ulva clathrata and Cladosiphon okamuranus Seaweeds to detemine their antiviral activity against NDV, since they are less cytotoxic than any other conventional drugs. The cytotoxicity, Antiviral activity and Virucidal activity was investigated by MTT assay, syncytia reduction assay and virucidal assay respectively. The results indicated a successful syncytia reduction assay as the SP inhibited syncytia formation by 67%, when added before F protein cleavage (Aguilar-Briseño et al., 2015).

In 2017, M. Shahid Mehmood and his coworkers from Institute of Microbiology at University of Agriculture Faisalabad used Azadirachta indica (Neem) Bark Extract against Newcastle Disease Virus in-vitro and in-ovo. The results indicate 75% reduced viral activity in micro-hemagglutination test but it leaves greater cytotoxic effects. The antiviral activity of neem bark extracts has shown promise, suggesting their potential as a viable alternative for antiviral treatment while also reducing their cytotoxic effects.

In 2018, Arif Ullah Khan and coworkers worked on the Nigella Sativa Extract against the Newcastle Disease Virus and evaluated its efficacy in a study conducted on experimentally infected chicken embryonated eggs. The researchers conducted experiments and found that

ethanolic extracts of Nigella sativa have shown significant effectiveness in reducing viral load and mortality caused by NDV in chicken embryos. There is a suggestion to incorporate Nigella sativa in the diet of broiler and laying hens, as well as administering its extract in their drinking water considering its potential to boost their resistance to most viral diseases and increase the profitability of poultry husbandry.

In 2019, Harazem and her coworkers tested extracts of Allium Cepa (Brown leaves of onion) and Allium Sativum (Bulbs of Garlic) against NDV lesions in 10 days old ECEs (Embryonated Chicken Eggs) at department of Virology, Mansoura University. The findings demonstrated the efficacy of garlic and onion extracts as inhibitors of NDV. However, the precise mechanism underlying this inhibitory impact remains unknown, necessitating more research and analysis into the antiviral properties of these extracts at various phases of the virus's reproduction cycle in tissue culture systems.

In 2020, Sekhar et. al tested the efficacy of newly designed and synthesized phosphorylated derivatives of abacavir through an experimental approach. The binding efficacy of the compounds with the HN protein of NDV was determined through molecular docking studies. The in-ovo, in-vitro and tissue culture results indicated prominent antiviral activity against NDV, and further studies are encouraged to determine in-depth antiviral efficacy (Chandra Sekhar et al., 2020).

In 2020, Antony, Kumar and the research team conducted an investigation into the potential efficacy of Nitazoxanide as a treatment for Newcastle disease virus, with a particular focus on its ability to modulate host cytokines. Nitazoxanide (NTZ) is classified as an antiparasitic medication and belongs to the thiazolide class of drugs. This study aimed to examine the impact of NTZ on the replication of NDV. The expression of NDV non-structural genes was analyzed that resulted in significant decrease in viral genes which further confirmed that viral

replication had been halted. The findings of the study suggested that Nitazoxanide (NTZ) had the potential to be utilized as a pharmaceutical agent with antiviral properties against Newcastle Disease Virus (NDV) (Antony et al., 2020).

In 2021, Chongyang Wang and his coworkers assessed the 1-Formyl-B-carboline derivatives in order to determine their efficacy in inhibiting the proliferation of the Newcastle disease virus. Specifically, the evaluation focused on the derivatives' ability to block the viral adsorption and entrance process. The experimental results suggested that the carboline derivatives had the ability to prevent the entry of NDV by interfering with the PI3K/Akt pathway, hence disrupting the early phases of the NDV life cycle (C. Wang et al., 2021).

2.6. Limitations of Currently Reported Therapeutics

Recent genotyping of NDV isolates suggests that vaccines and therapeutics against NDV developed till now are losing effectiveness against these 21st century viruses because of constant antigenic evolution. Despite the availability and extensive use of vaccines and therapeutics against NDV, significant outbreaks have still been observed all over the world. There is a pressing need for renewed research on NDV-induced immunity in poultry. Due to the capacity of virulent strains of Newcastle Disease Virus to cause high mortality, the virus continues to pose a threat to the global poultry industry. There can be certain factors which can be responsible for the limitation of the current available vaccines and therapeutics against NDV (Liu et al., 2017).

Some of the most common reasons which are responsible for the limitations of therapeutics involve, unequal mass administration, lack of efficacy since NDV possess strong antigenic shift and drift and evolves rapidly, potential development of drug resistance and lack of regulatory approvals.

One of the most prevalent restrictions is the costly and unpredictable nature of drug development, resulting in a significant number of potential medications failing to reach the market. Approximately 12% of pharmaceutical compounds that undergo clinical trials successfully obtain approval from the Food and Drug Administration (FDA) for commercial distribution. The development process frequently spans over a decade or longer, primarily due to the comprehensive procedures involved. These procedures encompass laboratory research and clinical trials of promising new drugs, as well as the allocation of resources towards drugs that do not progress beyond the laboratory-development stage, those that enter clinical trials but prove unsuccessful, or those that are withdrawn by the drug manufacturer for commercial reasons. Additionally, drugs that fail to obtain approval from the FDA also contribute to the extended duration of the development process. Additional obstacles include a scarcity of skilled workforce, challenges in managing the supply chain, and limitations in resources and infrastructure.

Therefore, diverse strategies are required to either prevent the spread of NDV or lessen its devastating impact on infected flocks. Advancements in bioinformatics and computational biology have enabled a greater level of understanding about therapeutics design. With the help of these tools, not only the specificity of drugs can be predicted easily, but also their target proteins can be evaluated. With the help of docking, potential drug targets can easily be identified through AutoDockVina by using computer aided tools.

2.7. Drug Repurposing

Lacking an effective antiviral treatment for Newcastle Disease Virus infection, a number of replicative cycle inhibitors are currently under investigation. Given the swift propagation of NDV and the typical duration involved in developing a new pharmaceutical product, the utilization of already licensed medications intended for different disorders to discover

innovative inhibitors for this viral sickness presents an appealing approach for expedited therapeutic intervention.

The primary aim of drug repurposing is to ascertain various targets for pre-existing pharmaceutical compounds. This branch of drug discovery is widely recognized as being of great importance due to its ability to address challenges related to drug optimization, development, and preclinical testing. The cost-effectiveness and expediency of repositioning already-approved medications can be attributed to the ready availability of pharmacological and toxicological data pertaining to these drugs. The utilization of prior investments is coupled with the reduction of clinical efforts, resulting in decreased costs, time, and failures commonly associated with the conventional drug discovery process. Furthermore, it provides an understanding of the matrix that represents the interaction between drugs and their respective targets. The utilization of high-performance computers for the purpose of secure virtual screening is a notable advantage of employing in silico approaches. The utilization of virtual screening is crucial in the large-scale manufacture of pharmaceuticals when dealing with extensive data sets. The utilization of molecular docking in computational biology is employed in the process of repurposing pharmaceuticals. Docking is a computational method used to anticipate the optimal spatial arrangement of one molecule in relation to another molecule, with the aim of generating a stable complex.

This approach additionally functions to figure out the deficiencies within the drug-target interaction matrix, consequently presenting information regarding the safety and effectiveness. The growth and progress of research on repurposing established drugs to treat diseases other than their original prescriptions have been encouraged by recent successes.

2.8. Repurposing of Drugs

2.8.1. Thiazolidinediones

Thiazolidinediones (TZDs) refer to synthetic exogenous agonists of the peroxisome proliferator-activated receptor-y (PPARy) that are employed in the management of type 2 diabetes. In the context of experimental animal models and persons receiving treatment with thiazolidinediones (TZDs), it has been observed that TZDs exhibit the ability to enhance insulin sensitivity, reduce blood glucose levels, suppress inflammation, and decrease blood pressure (Zou & Hu, 2013).

Pioglitazone, a member of the thiazolidinedione group with several pharmacodynamic properties, is an insulin sensitizer used for the treatment of diabetes mellitus (DM) (Richter et al., 2006).

It is orally bioavailable and has also been used in combination with other antiviral drugs against many viruses and cancer therapies. Prophylactic treatment of pioglitazone to A/PR/8 influenza virus-infected mice in experimental murine models has been shown to increase survival by 20-40% (Darwish et al., 2011). Another study reported use of pioglitazone against HIV-1 in EcoHIV infected mice models by successfully reducing inflammatory markers through downregulation of the HIV-1 (Omeragic et al., 2019). pioglitazone also presented synergistic antiviral effect against SARS-CoV-2 in Vero E6 cells (Imamura et al., 2021).

Englitazone, also a member of the thiazolidinedione family, is reported to be therapeutically effective by inhibiting the rate of gluconeogenesis from lactate and dihroxyacetone while successfully regulating glycolysis from lactate. But little information is available regarding direct effect of englitazone in glucose metabolism (Adams et al., 1998). It has also been reported in a computational analysis against SARS-CoV2 protein ligand interations but only

two of its amino acids were in common with the reference drugs taken, so it was not proposed for further invitro or invivo analysis.

Troglitazone, was the first drug to become widely available in United States, Europe and Japan after successful clinical trials resulting in lowered blood glucose concentrations. But later it was discovered that it has tendency to cause hepatic dysfunction, which led to the withdrawal of the drug in some countries (Imura, 1998). It has also been shown to inhibit HBV infection by preventing the hepatitis B virus internalization, a step-in cell cycle, via the dissociation of NTCP dimers on the plasma membrane (Fukano et al., 2019). Troglitazone has also been reported as a potential inhibitor along with four other drugs against SARS-CoV2 by interrupting NSP9 (non-structural replicase protein 9) binding with RNA or other proteins (Chandra et al., 2022).

Balaglitazone, is a partial agonist of PPARy of the second generation, with partial agonistic qualities. It was developed by Dr. Reddy's laboratories India and clinically tested against DM-II, reported by Henriksen et al. His report stated good safety profile of Balaglitazone at 10-20mg and positive effects on glycemic control parameters but weight gain and less fluid retention among experimental group was observed as a negative trend. To see if this holds true in a clinical situation, more study was required (Henriksen et al., 2011).

In 2017, Bahman and team reported a study in which Balaglitazone was used in doxorubicin resistant leukemic cells to reverse multidrug resistance mediated by P-glycoprotein. The results indicated downregulation of P-glycoprotein and significant improvement in cell viability followed by increasing doxorubicin levels within cells and decreased multidrug resistance (Yousefi et al., 2017).

Ralitoline, was the novel anticonvulsant thiazolidinedione derivative that had shown promising results in animal models of epilepsy in 90's. In terms of mechanism of action,

ralitoline's local anesthetic qualities were found to be responsible for its anticonvulsant activity since it inhibited the rapid sodium inward current in cultured heart ventricular cells (Fischer et al., 1992).

Etozoline, was used as an antihypertensive and diuretic drug in USA and Europe but now discontinued due to cardiac problems. No detailed data is available regarding this drug.

Lobeglitazone, a PPAR- agonist that demonstrated considerably better glucose control and a good effect on lipid profile, is primarily eliminated in the feces making bladder cancer less of a concern which is reported in other TZDs (Kim et al., 2015).

It was also reported in another study done by Juliyan and his team in which they observed role of lobeglitazone, In-silico, as HPV E1 protein inhibitor, highly conserved across all HPV types and is important for controlling how viruses replicate their DNA (Gunasinghe et al., 2023).

Rosiglitazone, oral antidiabetic drug that improved glycemic control in type 2 diabetic patients including its impact on glucose metabolism and insulin sensitivity. In 2007, Nissen and Wolski conducted a meta-analysis which revealed a significant association between rosiglitazone and an increased risk of myocardial infarction and cardiovascular-related mortality. Consequently, many nations have imposed restrictions on the use of this medication (Nissen & Wolski, 2007).

This drug was also reported by Jing Zhao and his coworkers, as EHV-1 inhibitor alongside quinacrine. The respiratory illness, abortion, and neurological abnormalities seen in horses are all caused by equine herpesvirus type 1 (EHV-1). EHV-1 infection occurs in both epithelial cells and CD172a+ monocytic cells inside the respiratory mucosa. Subsequently, these monocytic cells facilitate the transportation of the virus from the apical side of the epithelium to the lamina propria, finally leading to dissemination within the lymphatic and

bloodstream. The administration of RSG therapy did not demonstrate any significant impact on virus replication within the epithelial cells. However, it did result in a notable decrease in the quantity of CD172a+ cells that were infected within the lamina propria (Zhao et al., 2017).

Another study reported repositioning of rosiglitazone as anti-human adenovirus drug. HAdV infection negatively regulates the Type I interferon (IFNs) signaling pathway, while rosiglitazone boosts STAT1 phosphorylation, which is necessary for inducing an antiviral IFN response. Overall, the results of this trial support further investigation into the possible therapeutic use of this FDA-approved medicine in the treatment of HAdV infections (X. Wang et al., 2020).

Ciglitazone, has been reported against sepsis treatment by exhibiting anti-inflammatory properties as well as lowering neutrophil infiltration in the lung, colon, and liver and preventing the increase of cytokines in plasma, it also improves hypotension and vascular damage. Decreases in inflammation are likewise linked to considerable increases in survival rates (Zingarelli et al., 2003).

Ciglitazone is also reported to trigger cancer cell death via extrinsic and intrinsic receptor-dependent and/or -independent pathways in Ca Ski cells harboring Human Papillomavirus type 16 through PPARy-independent processes. For the first time it was found that ciglitazone inhibits the expression of E6, a viral oncoprotein known to disrupt the TRAIL pathway, leading to cell death. This data demonstrates that ciglitazone can counteract E6's anti-apoptotic effects and restore TRAIL's ability to kill cervical cancer cells (Plissonnier et al., 2017).

Teneligliptin, is a highly efficacious, specific, and durable inhibitor of dipeptidyl peptidase-4 (DPP-4) that is utilized in the management of Diabetes Mellitus II. It has received regulatory

approval in both Japan and Korea. Research is currently being undertaken in many nations as well. According to a computational research, there is evidence to show that it has the capacity to block the SARS-CoV-2 Mpro viral cysteine protease by reversible, noncovalent binding. Additionally, additional studies have indicated its potential in inhibiting the viral cysteine proteases SARS-CoV Mpro and MERS-CoV CLpro (P. P. N. Rao et al., 2021).

This study aims to investigate the drug repurposing approach as a primary strategy for addressing viral diseases, which pose significant challenges for the development of vaccines and therapeutics due to their genetically diverse genotypes that continuously evolve and have a wide geographical distribution. The study employed a systematic computational methodology to evaluate the potential of repurposed drugs that have previously demonstrated anti-viral, anti-cancer, anti-diabetic, anti-inflammatory, and cytotoxic properties. This assessment involved predicting the drug-likeness, oral bioavailability, efficacy, and toxicity risk of these drugs prior to conducting wet-lab experiments. The utilization of computational methods for the screening and prediction of repurposed pharmaceuticals, along with their favorable pharmacodynamic and pharmacokinetic qualities, offers significant advantages in terms of time efficiency and cost-effectiveness. The present investigation was undertaken with the objective of assessing the inhibitory capacity of these medications against four key structural proteins of the Newcastle disease virus, namely Fusion, Hemagglutinin-neuraminidase, Matrix, and Nucleocapsid. The research involved performing molecular docking and molecular dynamics (MD) simulations on ligand-protein complexes. The objective was to assess the stability of the docked structure. Additionally, several pharmacokinetic analyses were undertaken to evaluate the drug-likeness and toxicity potential of the medications. These analyses aimed to identify viable leads for the development of an anti-NDV therapy.
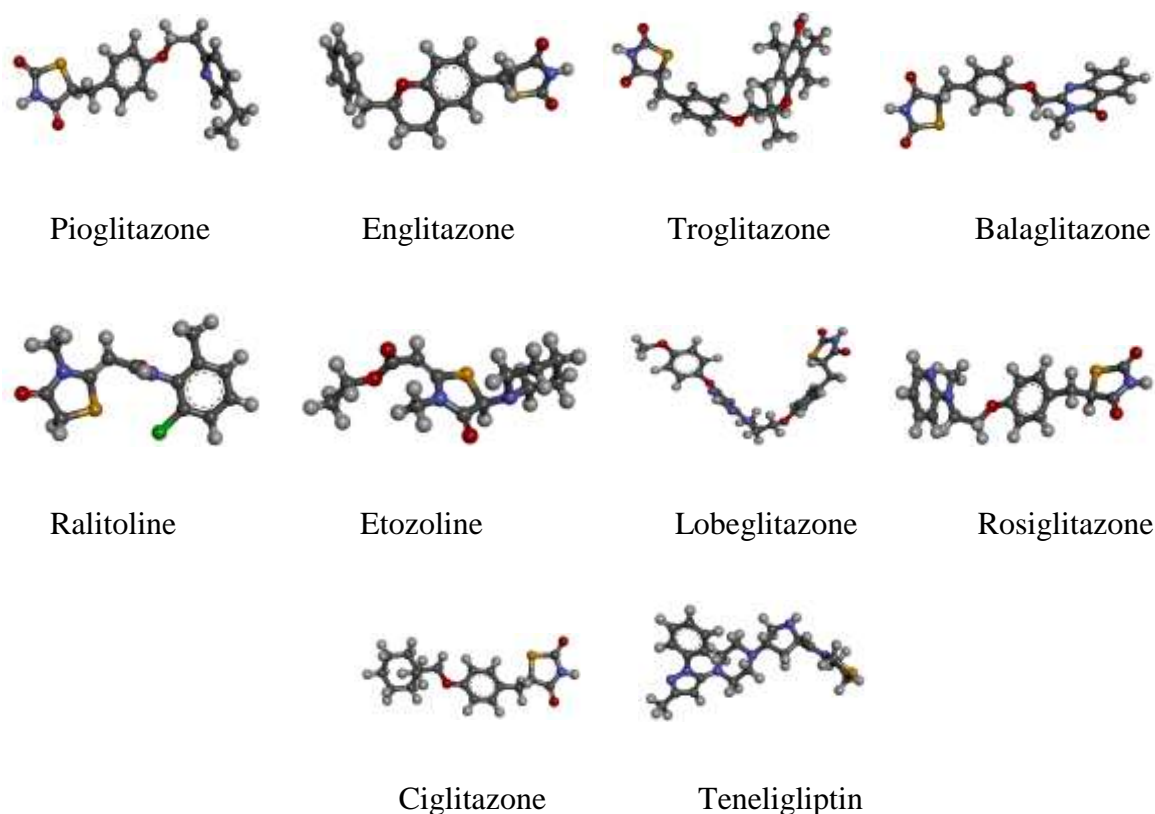
MATERIALS AND METHODS

3.1. Data Sources

This study was conducted at Vaccine and Therapeutic Development Lab, Atta Ur Rahman School of Applied Biosciences, National University of Sciences and Technology. A total number of ten commercially available Thiazolidinedione based drugs were selected from literature. The selection criteria were based on their respective structure activity relationship.

3.2. Ligand Selection

For the present study all commercially available thiazolidinedione-based drugs reported in literature were selected for ligand preparation. The selected drugs were Pioglitazone, Englitazone, Troglitazone, Balaglitazone, Ralitoline, Etozoline, Lobeglitazone, Rosiglitazone, Ciglitazone and Tenegliptin. 3D structures of the selected molecules were retrieved from Pub-Chem (**https://pubchem.ncbi.nlm.nih.gov/**) in SDF format. Subsequently, PyMol software was employed to transform these structures into PDB format. The drugs were prepared for docking in AutoDock Vina by applying charge fixation and polar hydrogen addition. The resulting prepared drugs were saved in the PDBQT file format. The physicochemical parameters and molecular structures of the chosen Thiazolidinedione based drugs are provided in Table 3.1, while the three-dimensional structures can be observed in Figure 3.1.

Table 3.1: Physicochemical properties of selected Thiazolidinedione based drugs.

S. No.	Drugs	PubChem CID	Molecular Formula	Molecular Weight	Chemical class of drugs
1.	Pioglitazone	CID_4829	$C_{19}H_{20}N_2O_3S$	356.4 g/mol	Thiazolidinedione (TZD)
2.	Englitazone	CID_60303	$C_{20}H_{19}NO_3S$	353.4 g/mol	Thiazolidinedione (TZD)
3.	Troglitazone	CID_5591	$C_{24}H_{27}NO_5S$	441.5 g/mol	Thiazolidinedione (TZD)
4.	Balaglitazone	CID_9889200	$C_{20}H_{17}N_3O_4S$	395.4 g/mol	Thiazolidinedione (TZD)
5.	Ralitoline	CID_6436118	$C_{13}H_{13}ClN_2O_2S$	296.7 g/mol	Thiazolidinedione (TZD)
6.	Etozoline	CID_5743585	$C_{13}H_{20}N_2O_3S$	284.3 g/mol	Thiazolidinedione (TZD)
7.	Lobeglitazone	CID_9826451	$C_{24}H_{24}N_4O_5S$	480.5 g/mol	Thiazolidinedione (TZD)
8.	Rosiglitazone	CID_77999	$C_{18}H_{19}N_3O_3S$	357.4 g/mol	Thiazolidinedione (TZD)
9.	Ciglitazone	CID_2750	$C_{18}H_{23}NO_3S$	333.4 g/mol	Thiazolidinedione (TZD)
10.	Teneligliptin	CID_11949652	$C_{22}H_{30}N_6OS$	426.6 g/mol	Dipeptidyl peptidase-4 inhibitors

Pioglitazone   Englitazone   Troglitazone   Balaglitazone

Ralitoline   Etozoline   Lobeglitazone   Rosiglitazone

Ciglitazone   Teneligliptin

Figure 3.1: 3D structures of selected Thiazolidinedione based drugs.

3.3. Receptor Preparation

The 3D crystal structures of the structural proteins of Newcastle Disease Virus, namely the Fusion protein (F, PDB ID: 3MAW), Haemagglutinin-neuraminidase (HN, PDB ID: 4FZH), Matrix protein (M, PDB ID: 4G1L) and Nucleocapsid protein (NP, PDB ID: 6JC3) were already available in RCSB database, (https://www.rcsb.org/) which were then obtained from Protein Data Bank in PDB format, represented in Figure 3.2. The data obtained from PDB was subsequently imported into Discovery Studio software in order to eliminate the initial ligands and other small molecules that were bound to the protein structures. Prior to conducting docking analysis, the 3D protein structures underwent refinement using AutoDock Vina. The process of refining the chosen proteins involved the removal of unnecessary ions, ligands (if present), and water molecules. In addition, the receptors were

modified by incorporating polar hydrogen atoms and Kollman charges, after which they were

saved in PDBQT format to facilitate the docking process.

      (a)   (b)

(c)   (d)
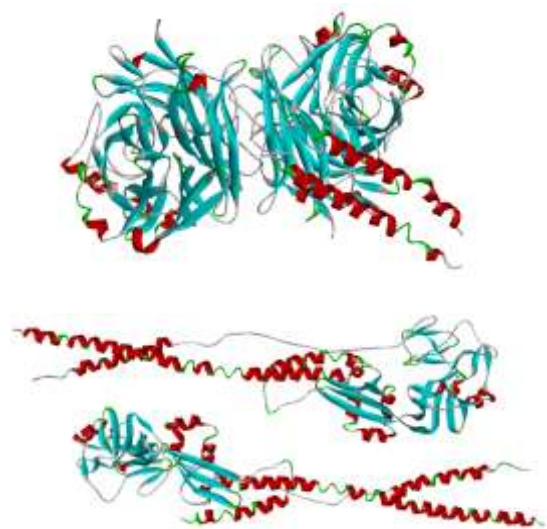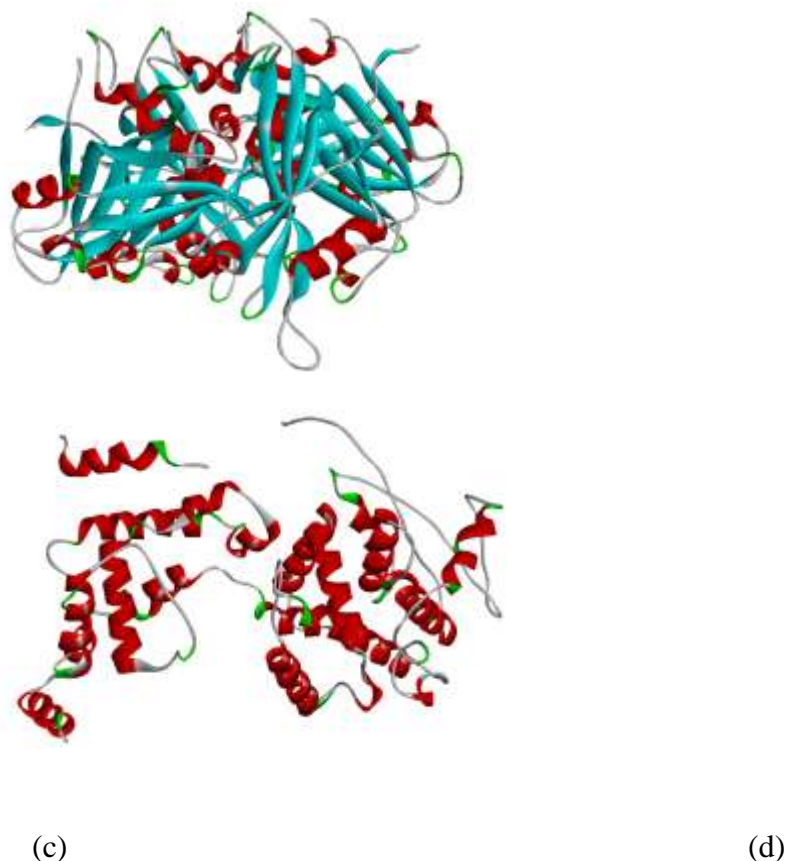
Figure 3.2: Visual representation of the 3D crystal structures of four Newcastle disease virus structural proteins. (a) Haemagglutinin-neuraminidase protein, (b) Fusion protein, (c) Matrix protein and (d) Nucleocapsid protein.

3.4. Molecular Docking

The docking of ligands, classified as "flexible", with protein targets, classified as "rigid", was performed using AutoDock Tools 1.5.6. AutoDock Vina is a user-friendly software tool that offers practical utility, efficient computational speed, and automated determination of grid box size, hence facilitating the convenient estimation of binding sites. The current work employed a grid box size of 40 x 40 x 40 (x, y, and z) and a grid spacing of 0.375 Å for all the receptor proteins. The coordinates for the grid center of the Fusion protein (F, PDB ID: 3MAW) were determined to be 18.565, -32.141, and -95.236. similarly, for the

Haemagglutinin-neuraminidase protein (HN, PDB ID: 4FZH) -11.751, -55.819, and -3.680, for Matrix protein (M, PDB ID: 4G1L) 32.229, 21.695, and -2.014 and for Nucleocapsid protein (NP, PDB ID: 6JC3) 41.432, 23.805, and 22.369, for x, y, and z coordinates respectively. A configuration file was generated for each ligand, encompassing the properties of the grid box. These files were produced in .txt format. In order to initiate the docking analysis, a series of instructions were developed and executed in the command line to provide the resulting score. The output file presented the binding affinity/gibbs free energy (ΔG) values in kcal/mol. The ligand exhibiting the most negative value was deemed to possess the greatest binding affinity towards a specific target protein. The model with the highest binding energy, specifically model 1, was chosen as the most optimal fit among the nine conformations that were created. The docking interactions between the selected ligands and the targeted molecules are depicted in Table 3.2.

Table 3.2: Docking Interactions of selected Thiazolidinedione based drugs with targeted proteins.

S. No.	Ligand	Receptor	Binding Affinity	Interacting Amino Acid Residues

			(kcal/mol)	
1.	Pioglitazone	HN Protein	-8.2	Thr222, Phe219, Leu160, Arg217
		Fusion Protein	-6.2	Glu209, Val63, Tyr213, Phe221
		Matrix Protein	-8.1	Ser223, Leu220, Lys158, Ile25
		Nucleocapsid Protein	-6.4	Asp 63, Asn65, Phe128, Leu68, Pro36, Val33
2.	Englitazone	HN Protein	-9.1	Ser221, Leu228, Ala214, Ile226, Arg211
		Fusion Protein	-7.2	Asn68, Val63, Val220
		Matrix Protein	-7.4	Gln40, Ser17, Tyr359, Arg42, Thr315
		Nucleocapsid Protein	-7.3	Ser246, Tyr248, Leu162, Thr247
3.	Troglitazone	HN Protein	-9.2	Thr222, Tyr204, Leu160, Ser221, Phe219
		Fusion Protein	-7.0	Glu209, Tyr213, Leu217, Val63
		Matrix Protein	-7.2	Ser150, Asn146, Cys93, Leu274, Lys188, Arg126
		Nucleocapsid Protein	-8.6	Lys178, Gly263, U38, Phe268, Thr265
4.	Balaglitazone	HN Protein	-8.9	Cys123, Ser225, Tyr204, Leu160, Tyr204, Thr222
		Fusion Protein	-7.3	Asn240, Val220, Phe221, Ile62
		Matrix Protein	-8.3	Pro219, Lys222, Gln310, Ser223
		Nucleocapsid Protein	-7.5	Gly315, Lys236, Arg237, Leu314
5.	Ralitoline	HN Protein	-7.3	Thr222, Pro164, Phe162

		Fusion Protein	-5.6	Tyr239, Glu216, Leu217, Val220
		Matrix Protein	-7.5	Pro219, Glu161, Ile314, Lys158
		Nucleocapsid Protein	-6.4	Thr265, Ala266, Thr331, Tyr348, Phe350, Pro324
6.	Etozoline	HN Protein	-7.0	Leu160, Thr222
		Fusion Protein	-5.0	Val220, Lys64, Pro64, Tyr213
		Matrix Protein	-7.3	Ser223, Gln310, Leu220, Ile25
		Nucleocapsid Protein	-5.5	Thr185, Ala266, Tyr348
7.	Lobeglitazone	HN Protein	-7.9	Tyr204, Pro164, Asn161, Ser221, Thr222
		Fusion Protein	-7.3	Lys64, Pro67, Val220, Glu216
		Matrix Protein	-7.1	Ser150, Arg126, Ser305, Leu274, Lys155
		Nucleocapsid Protein	-6.3	Ala266, Thr265, Lys347, Tyr 348, Glu188
8.	Rosiglitazone	HN Protein	-8.0	Asn227, Arg217, Val218, His202, Asp229, Phe219
		Fusion Protein	-5.6	Ile62, Phe221, Val220, Gln236
		Matrix Protein	-8.4	Lys158, Leu220, Ser223, Lys222
		Nucleocapsid Protein	-6.5	Lys178, Ala182, Gly263, Thr265
9.	Ciglitazone	HN Protein	-7.4	Ser225, Arg224, Pro164, Tyr204, Leu160
		Fusion Protein	-6.7	Tyr213, Val63, Phe221, Gln236
		Matrix Protein	-8.1	Lys158, Ile25, Leu220
		Nucleocapsid Protein	-6.5	Thr185, Arg192, Ala266, Tyr348

10.	Teneligliptin	HN Protein	-9.1	Thr222, Leu160, Pro164, Leu223
		Fusion Protein	-6.4	Ile62, Lys64, Asn240, Glu216
		Matrix Protein	-6.6	Ser150, Tyr148, Cys93, Leu274
		Nucleocapsid Protein	-7.0	Leu225, Ile229, Leu314

3.5. Prediction of Activity Spectra for Substances (PASS) Analysis

Prediction of Activity Spectra for Substances (PASS) analysis program was utilized to forecast several characteristics of substances, including biological qualities, pharmacological properties, drug-likeness, likely side effects, and mode of action. These predictions were made by examining the structure-activity relationship between the under-investigation ligands and the existing receptor molecule. The present study employed a range of online and offline methods to conduct PASS analysis, as outlined below:

3.6. Lipinski's Rule of Five

Lipinski's rule of five elucidates the molecular characteristics of pharmaceutical drugs by considering crucial pharmacokinetic factors, including absorption, metabolism, distribution, and excretion. The drug-likeness of the ten Thiazolidinedione based drugs included in the study was assessed utilizing Lipinski's rule of five. The drug-likeness of ligands was determined using the web program Swiss-ADME, which may be accessed at http://www.swissadme.ch/index.php. The drug-likeness of the compounds was assessed using several parameters, including the logarithm of the partition coefficient between n-octanol and water ($logP \leq 5$), molecular weight (MW ≤ 500), number of hydrogen bond donors (NOHNH

$\leq$ 5), number of hydrogen bond acceptor sites (NON $\leq$ 10), topological polar surface area (TPSA $\leq$ 140 Å2), and number of rotatable bonds ($\leq$ 10). In order to preserve the bioavailability of an orally active medicine, it is imperative that it does not exceed a single Lipinski violation.

3.7. Pharmacokinetic Property Prediction

The Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of the ligands under investigation were once again predicted using the online software Swiss-ADME (http://www.swissadme.ch/index.php). The pharmacokinetic characteristics of 10 Thiazolidinedione based drugs were examined, including their blood-brain barrier (BBB) permeability, distribution, gastrointestinal absorption, metabolism as a substrate for P-glycoprotein (P-gp), and their interaction with Cytochrome P450 enzymes (specifically CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). Additionally, the lipophilicity of these ligands was assessed in relation to their absorption through the plasma membrane.

3.8. Bioactivity Score Prediction

The drug score values serve as a measure of the inherent capacity of a possible compound to be considered as a viable candidate for medication development. The bioactivity scores of Thiazolidinedione based drugs against several chicken receptors, including G protein-coupled receptors (GPCRs), kinases, proteases, ion channels, enzymes, and nuclear receptors, were predicted using the web-based tool Molinspiration version 2021.03, which may be accessed at https://molinspiration.com/cgi-bin/properties. If the bioactivity score exceeds 0.0, the compound is classified as dynamic (active). If the score is between the range of -5.0 to 0.0, the complex is categorized as moderately active. Conversely, if the bioactivity value is below -5.0, it is deemed to be inert.

3.9. Toxicity Potential Study

The process of toxicity risk assessment provides preliminary data regarding the potential adverse effects of ligands that may be utilized in the field of drug discovery and development. The study employed the OSIRIS Data Warrior V5.2.1 software to analyze drug-likeness and assess the risk of drug toxicity. This involved evaluating many features, including drug-likeness, tumorigenicity, mutagenicity, reproductive effects, and irritating effects. The aim was to estimate the potential of novel drug candidates during the early stages of research.

3.10. Molecular Dynamic Simulations

The protein flexibility of the most optimal docked structures was assessed by molecular dynamic simulations using the CABS-flex 2.0 server (http://biocomp.chem.uw.edu.pl/CABSflex2). The resulting data was presented in terms of the root mean square fluctuation (RMSF) value. The simulations in CABS-flex were performed utilizing the default parameters, specifically: a protein rigidity value of 1.0, a total of 50 cycles, 50 cycles between each trajectory, a temperature range of 1.40, and a random number generator seed of 5711. The CABS-flex method provides efficient and accurate protein flexibility simulation with a high temporal precision of 10 nanoseconds, while significantly   minimizing   system   restrictions.

RESULTS

4.1. Molecular Docking Analysis

The docking of target proteins i.e., a) Haemagglutinin-neuraminidase (HN, PDB ID: 4FZH), b) Fusion protein (F, PDB ID: 3MAW), c) Matrix protein (M, PDB ID: 4G1L) and d) Nucleocapsid protein (NP, PDB ID: 6JC3). with 10 Thiazolidinedione based drugs was performed using AutoDock Vina version 1.5.6. These drugs exhibited distinct interactions within the binding pockets, demonstrating varying binding affinities towards the targeted proteins.

Out of 10 selected Thiazolidinedione based drugs, 6 drugs showed the highest negative binding energy with 4 targeted receptor molecules, represented in table 4.1.
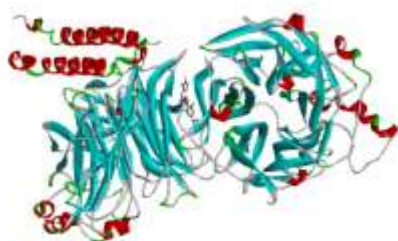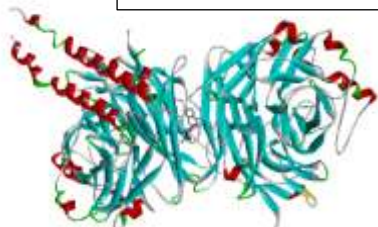
4.1.1. HN Protein Docking Complex with Drugs

HN/Englitazone (-9.1 kcal/mol)

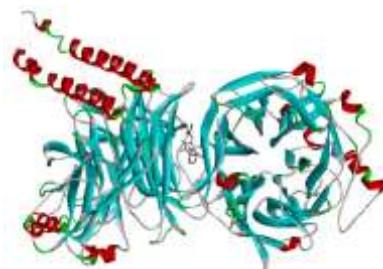HN/Troglitazone (-9.2 kcal/mol)
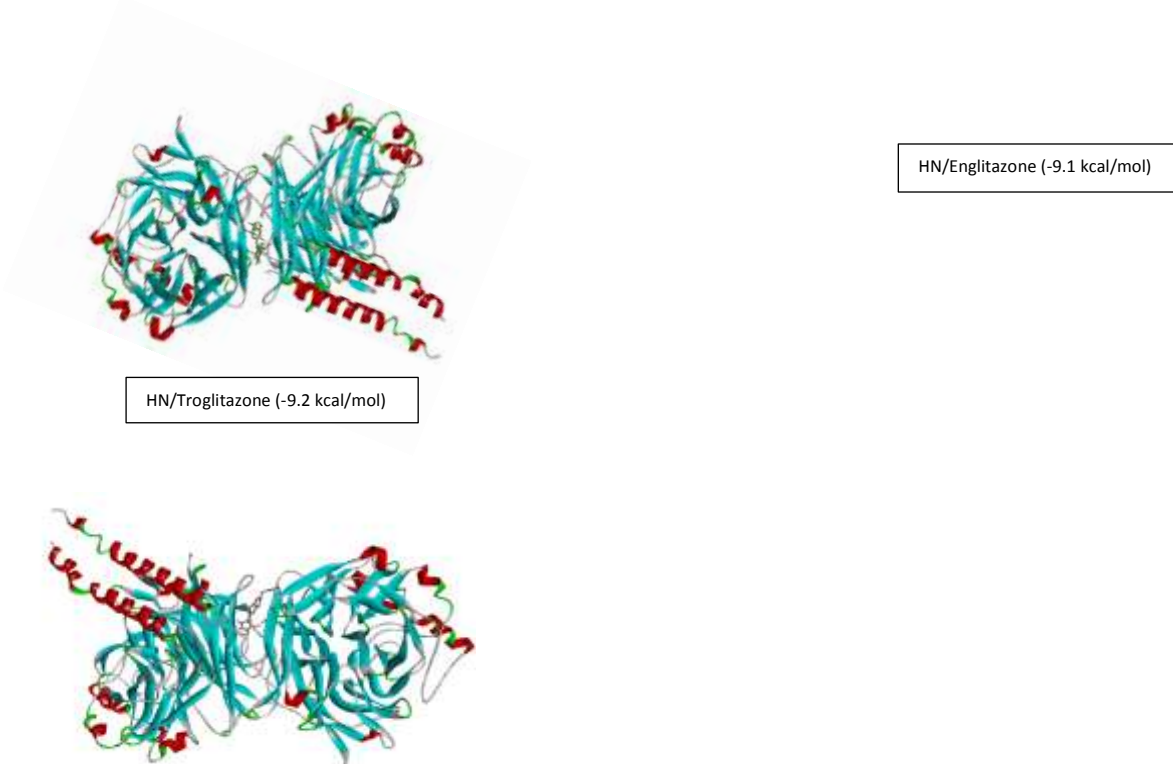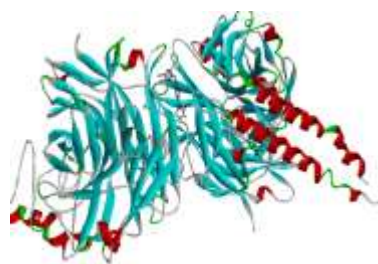
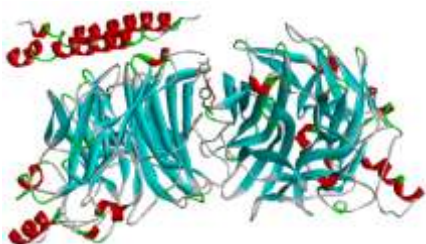HN/Pioglitazone (-8.2 kcal/mol)
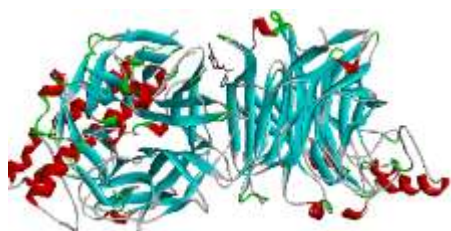
HN/Balaglitazone (-8.9 kcal/mol)
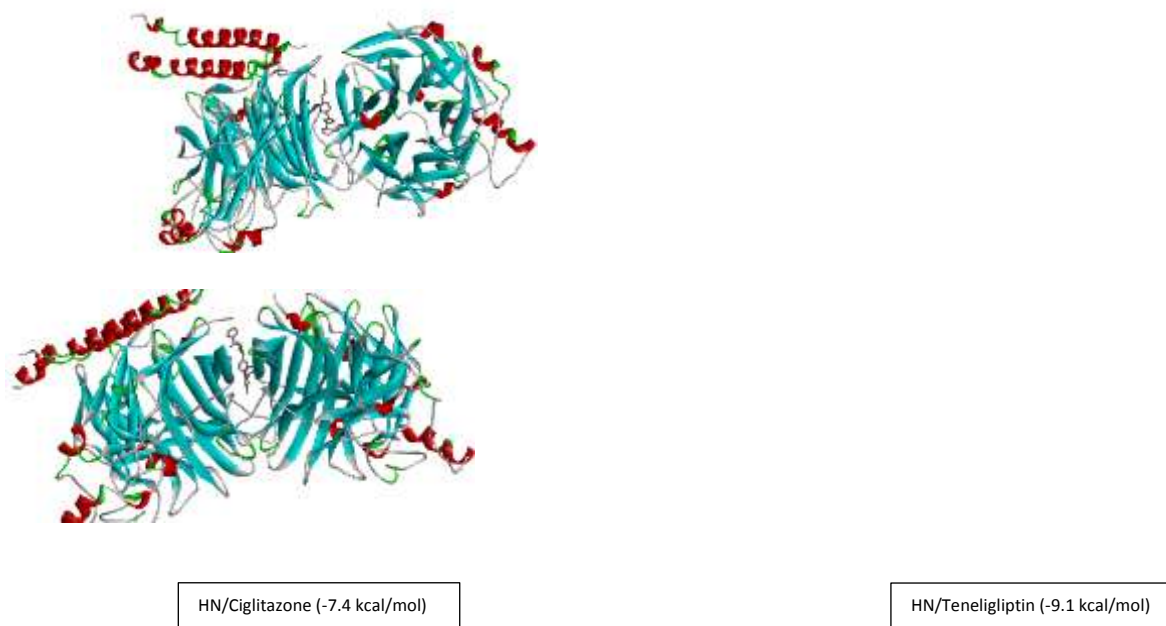
HN/Ralitoline (-7.3 kcal/mol)

HN/Etozoline (-7.0 kcal/mol)

HN/Rosiglitazone (-8.0 kcal/mol)

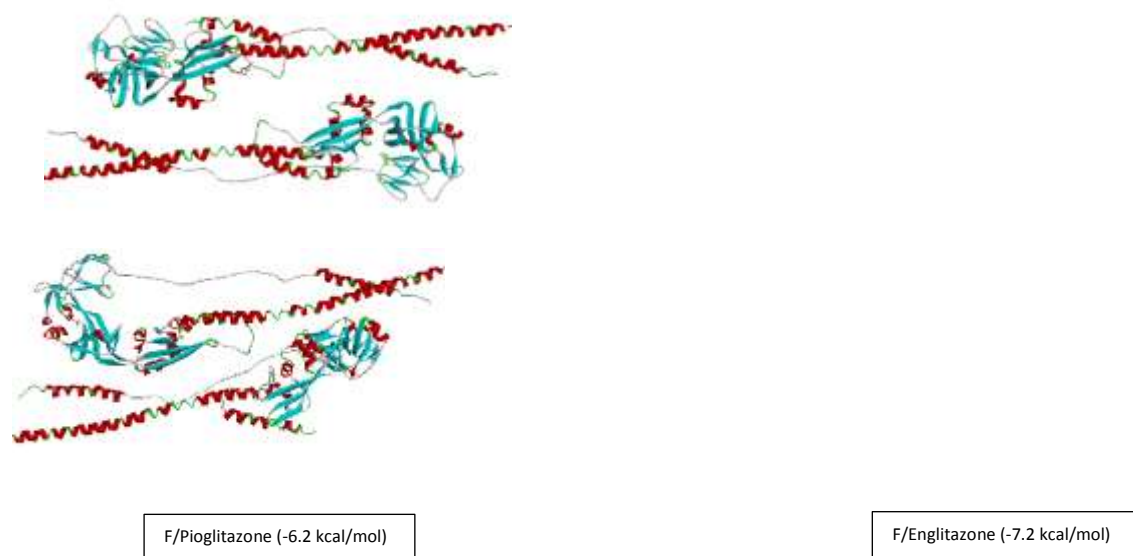HN/Lobeglitazone (-7.9 kcal/mol)

HN/Ciglitazone (-7.4 kcal/mol)
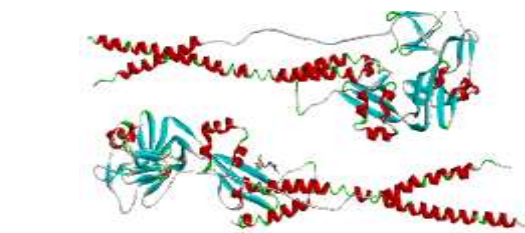
HN/Teneligliptin (-9.1 kcal/mol)

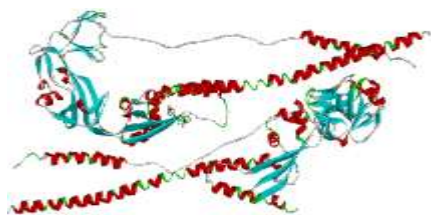**Figure 4.1:** Visual representation of Haemagglutinin-neuraminidase (HN, PDB ID: 4FZH) protein docking complex with 10 Thiazolidinedione based drugs.

4.1.2. Fusion Protein Docking Complex with Drugs:

F/Pioglitazone (-6.2 kcal/mol)

F/Englitazone (-7.2 kcal/mol)
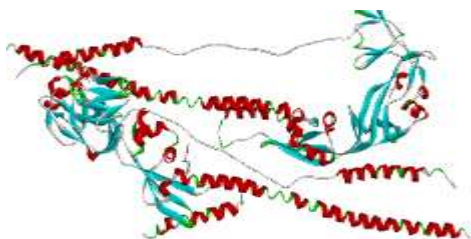
F/Troglitazone (-7.0 kcal/mol)

F/Balaglitazone (-7.3 kcal/mol)

F/Ralitoline (-5.6 kcal/mol)

F/Etozoline (-5.0 kcal/mol)

F/Lobeglitazone (-7.3 kcal/mol)

F/Rosiglitazone (-5.6 kcal/mol)

F/Ciglitazone (-6.7 kcal/mol)

F/Teneligliptin (-6.4 kcal/mol)

Figure 4.2: Visual representation of Fusion protein (F, PDB ID: 3MAW) docking complex with 10 Thiazolidinedione based drugs.

4.1.3. Matrix Protein Docking Complex with Drugs

M/Pioglitazone (-8.1 kcal/mol)
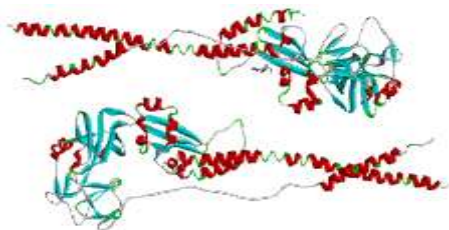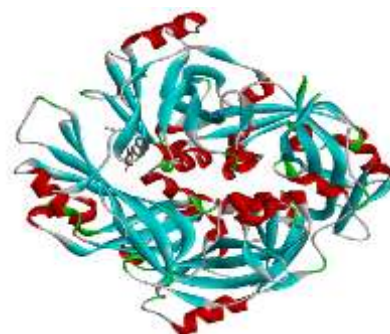

M/Englitazone (-7.4 kcal/mol)
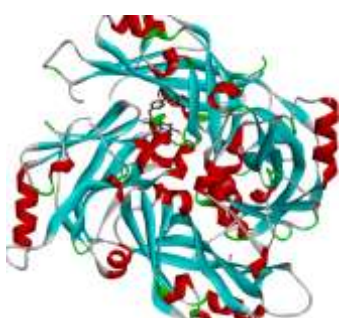
M/Troglitazone (-7.2 kcal/mol)
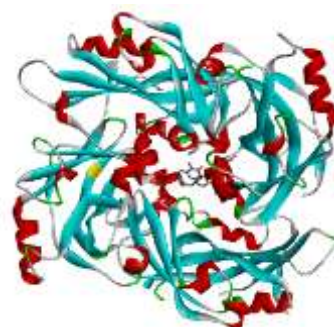
M/Balaglitazone (-8.3 kcal/mol)
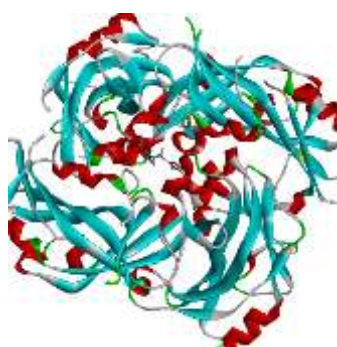
M/Ralitoline (-7.5 kcal/mol)
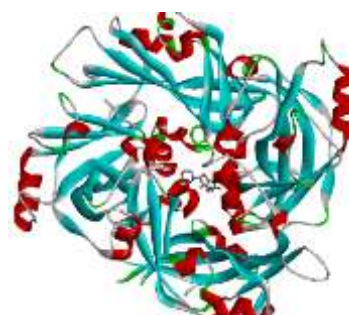
M/Etozoline (-7.3 kcal/mol)
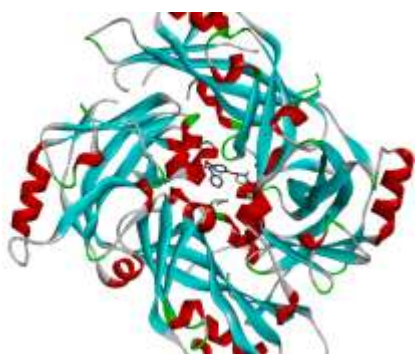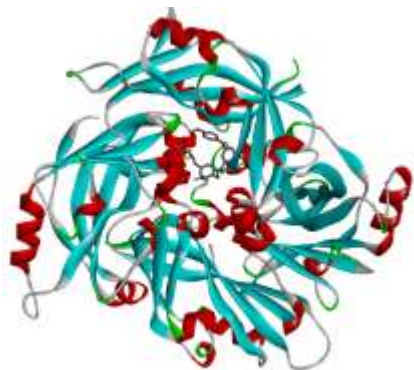
M/Lobeglitazone (-7.1 kcal/mol)

M/Rosiglitazone (-8.4 kcal/mol)

M/Ciglitazone (-8.1kcal/mol)

M/Tenegliptin (-6.6 kcal/mol)
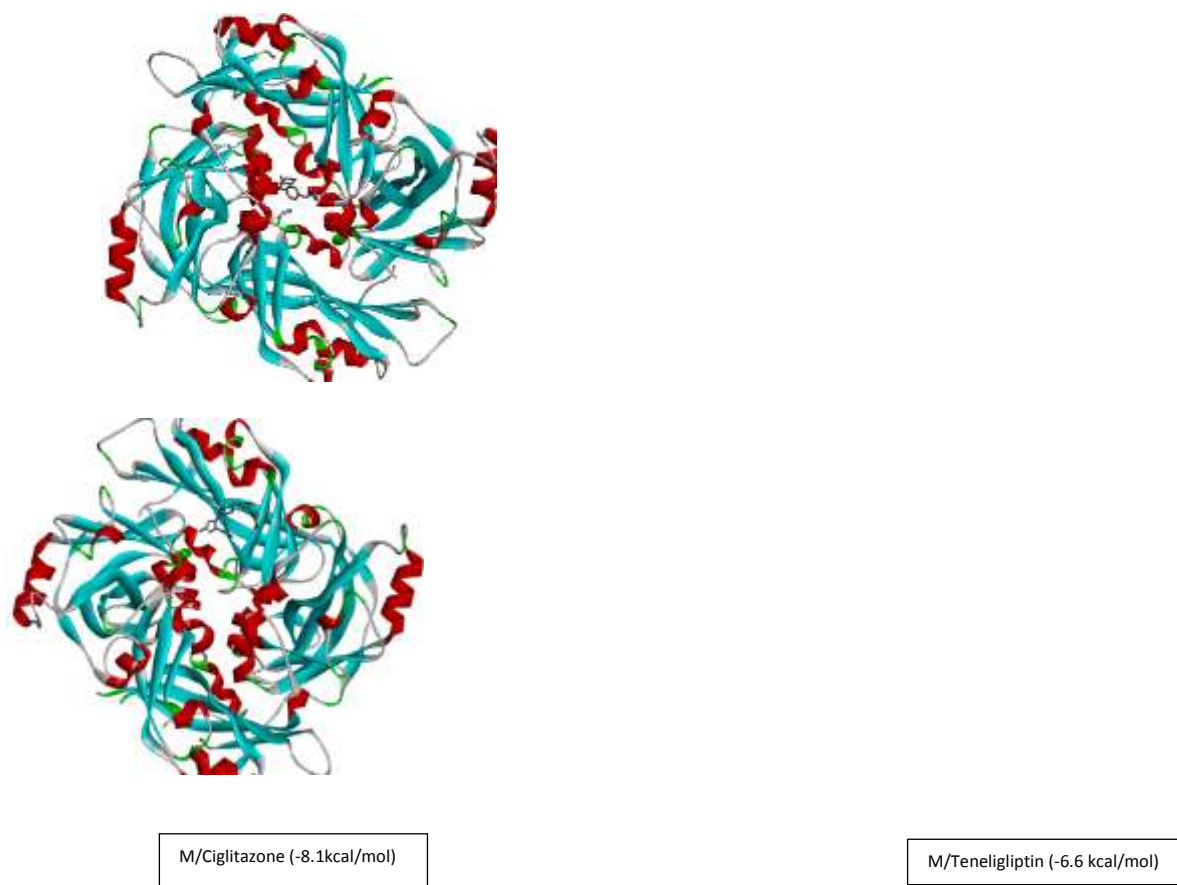
Figure 4.3: Visual representation of Matrix protein (M, PDB ID: 4G1L) docking complex with 10 Thiazolidinedione based drugs.

4.1.4. Nucleocapsid Protein Docking Complex with Drugs
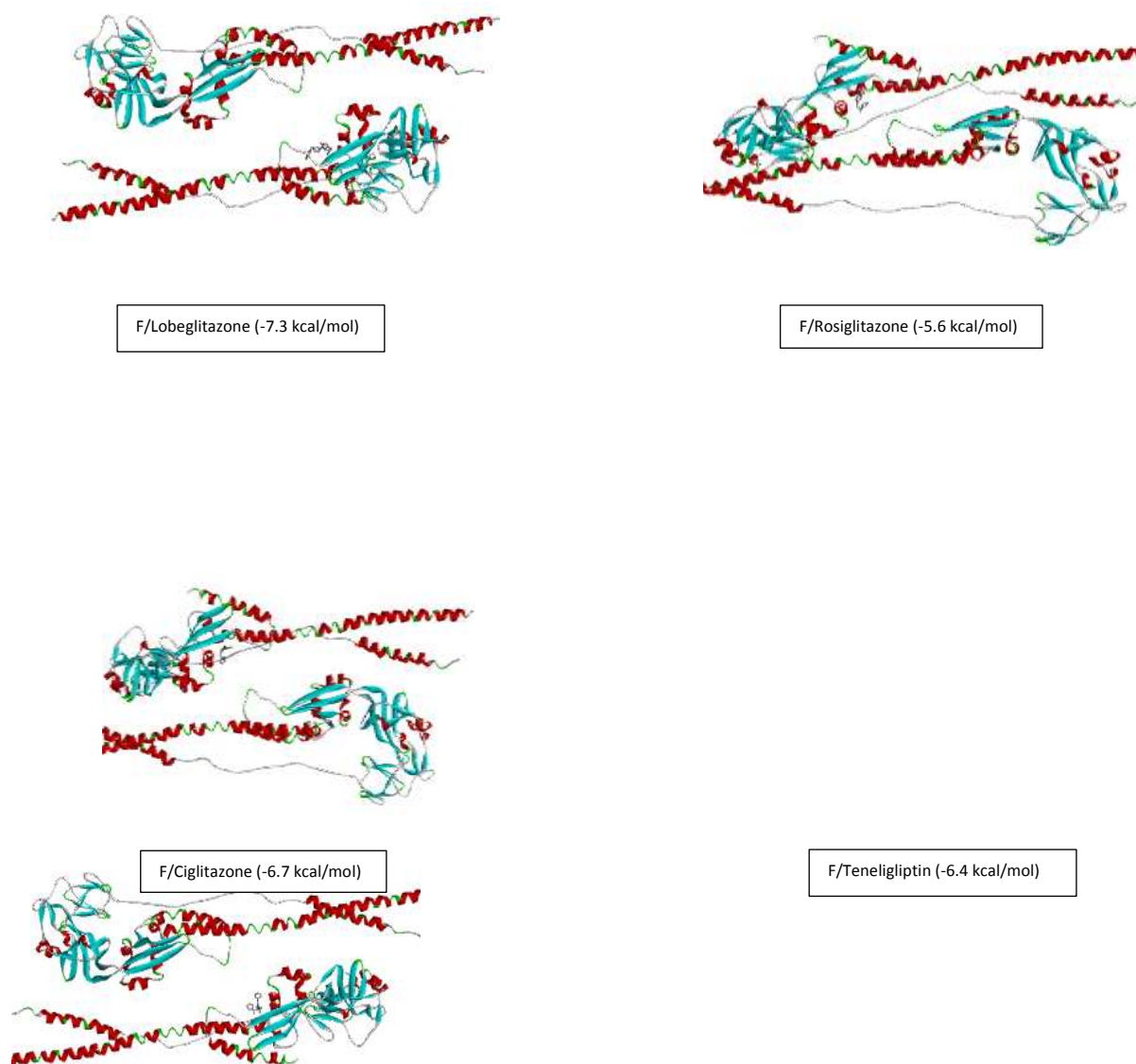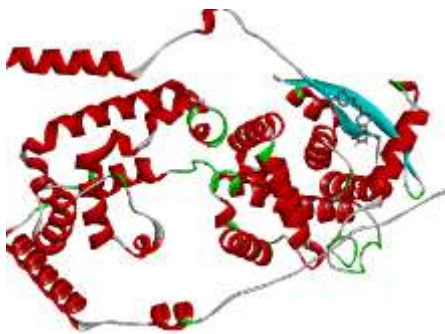
NP/Pioglitazone (-6.4 kcal/mol)

NP/Englitazone (-7.3 kcal/mol)
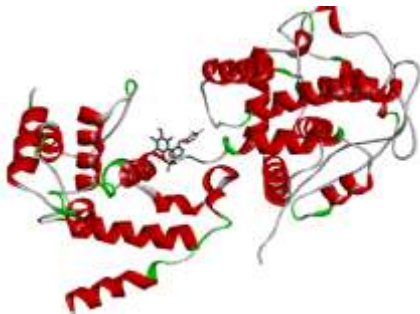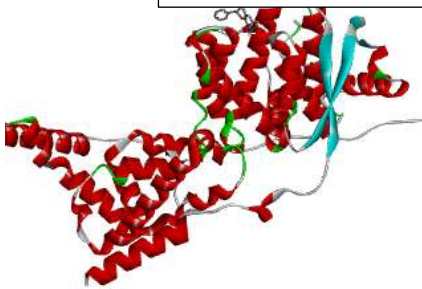
NP/Troglitazone (-8.6 kcal/mol)

NP/Balaglitazone (-7.5 kcal/mol)
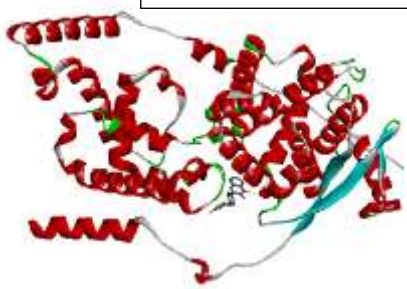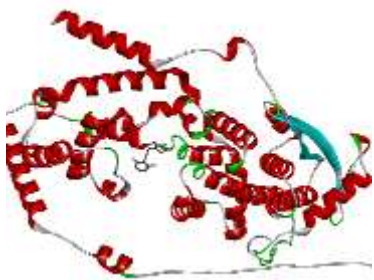
NP/Ralitoline (-6.4 kcal/mol)
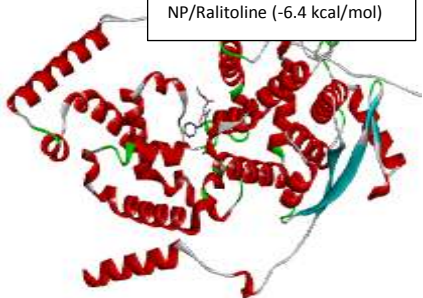
NP/Etozoline (-5.5 kcal/mol)
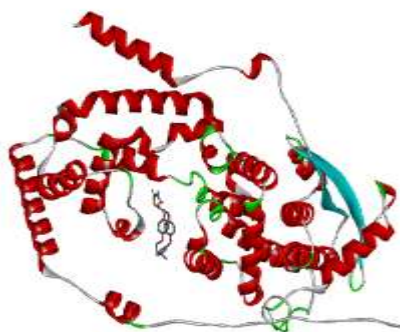
NP/Lobeglitazone (-6.3 kcal/mol)

NP/Rosiglitazone (-6.5 kcal/mol)
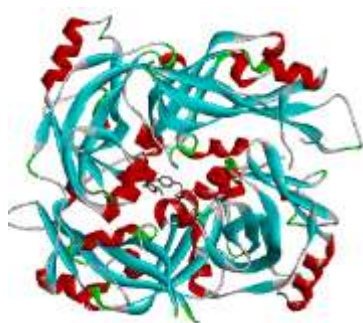
NP/Ciglitazone (-6.5 kcal/mol)
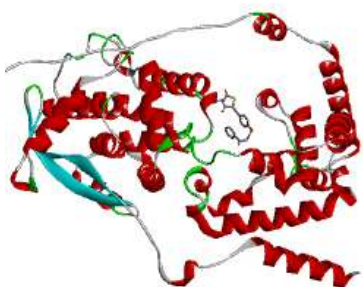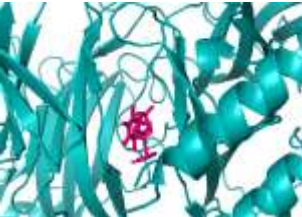
NP/Tenegliptin (-7.0 kcal/mol)
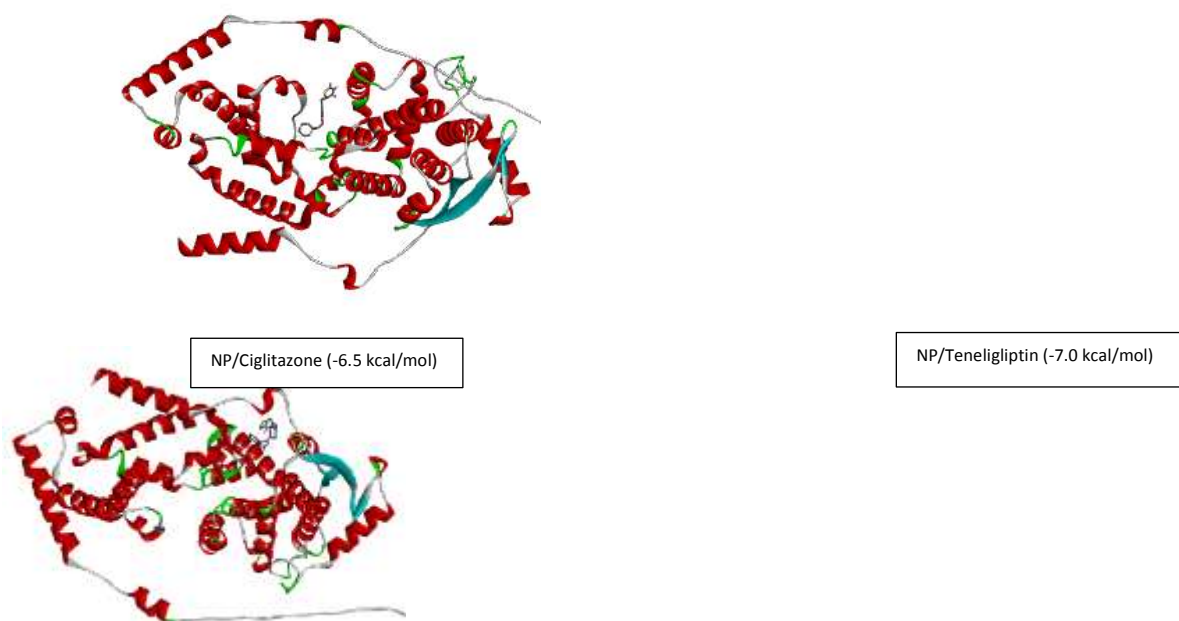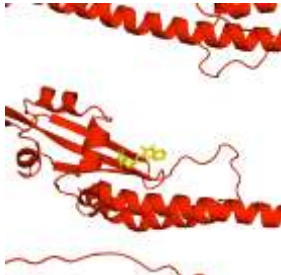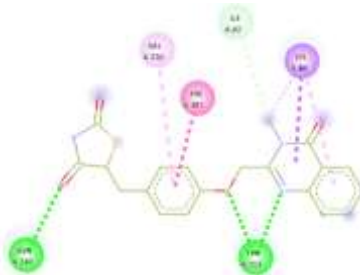
Figure 4.4: Visual representation of Nucleocapsid protein (NP, PDB ID: 6JC3) docking complex with 10 Thiazolidinedione based drugs.

Table 4.1: Docking interactions of 6 Thiazolidinedione based drugs of highest binding energy with targeted receptor molecules.

Sr. No.	Ligands	Receptors	Binding Energy Kcal/mol	Visual Representation	Interacting Amino Acids
1.	Troglitazone	HN protein	-9.2		
2.	Englitazone	HN protein	-9.1		
3.	Troglitazone	Nucleocapsid protein	-8.6		

4.	Rosiglitazone	Matrix protein	-8.4		
5.	Ciglitazone	Matrix protein	-8.1		
6.	Balaglitazone	Fusion protein	-7.3		

4.2. Lipinski's rule of five

Lipinski's rule highlights the molecular attributes of a compound that are of utmost importance in the process of lead optimization and the attainment of selectivity for a potential orally administered therapeutic candidate over the course of clinical trials. Typically, a pharmacologically active compound administered orally should adhere to the Lipinski's rule of five, wherein the presence of no more than one violation is preferred to maintain optimal bioavailability. The physicochemical properties of ten drugs based on Thiazolidinedione were

calculated using PASS analysis, incorporating Lipinski's rule of five. All of the ligands that were chosen did not show any violations of Lipinski's rule. All of the compounds that were chosen met the established criteria.

Table 4.2: PASS analysis (Lipinski's rule of five) of selected Thiazolidinedione based drugs.

S. No.	Ligands	cLog P (≤5)	MW (≤500 g/mol)	noHNH (≤5)	nON (≤10)	TPSA (≤140 Å2)	Number of Rotatable Bonds (≤10)	Lipinski's Violation
1.	Pioglitazone	3.09	356.44	1	4	93.59	7	0
2.	Englitazone	3.52	353.43	1	3	80.70	4	0
3.	Troglitazone	4.12	441.54	2	5	110.16	5	0
4.	Balaglitazone	2.37	395.43	1	5	115.59	5	0
5.	Ralitoline	2.15	296.77	1	2	74.71	3	0
6.	Etozoline	1.44	284.37	0	4	75.15	4	0
7.	Lobeglitazone	3.13	480.54	1	7	128.18	10	0
8.	Rosiglitazone	2.36	357.43	1	4	96.83	7	0
9.	Ciglitazone	3.62	333.45	1	3	80.70	5	0
10.	Teneligliptin	1.76	426.58	1	4	81.94	5	0

4.3. Pharmacokinetic property prediction

In order to evaluate the pharmacokinetic viability of several Thiazolidinedione-based drugs as potential candidates for repurposed therapeutic use, their ADMET characteristics, encompassing absorption, distribution, metabolism, excretion, and toxicity, were evaluated through the utilization of Swiss ADME software available online. The Log P o/w value,

which represents the consensus lipophilicity, was computed in order to assess the lipophilic characteristics of the chosen ligands. The analysis revealed that all of the compounds exhibited a lipid soluble nature. The results of the ADMET analysis conducted on the studied ligands indicate that, with the exception of Ralitoline, none of the ligands demonstrated the ability to permeate the blood-brain barrier. Among the ligands examined, Troglitazone was the sole compound that exhibited positive outcomes as a substrate for permeability glycoprotein (P-gp). Conversely, the remaining ligands yielded negative findings in this regard. The findings of this study indicate that the ligands, due to their non-P-gp substrate nature, exhibit prolonged cellular persistence, hence enhancing their pharmacokinetic efficacy. The phrase "skin permeability" (Kp) is employed to quantitatively measure the rate at which chemicals penetrate the outermost layer of the skin, known as the epidermis. Remarkably, each of the ten ligands had negative Kp values, suggesting a diminished likelihood of topical absorption for these ligands.

The cytochrome P450 (CYP) enzymes are a prominent superfamily of metabolic enzymes that play a crucial role in the biotransformation of xenobiotics. These compounds have the ability to function as substrates or inhibitors, and they play a crucial role in the metabolic processes of a wide range of pharmaceutical substances. The act of inhibiting the five classes of cytochrome P450 enzymes, specifically CYP3A4, CYP1A2, CYP2C9, CYP2C19, and CYP2D6, leads to elevated plasma concentrations, hence resulting in an augmentation of bioavailability. In order to achieve sustained plasma concentrations and improved bioavailability, the ligands being studied were anticipated to exert inhibitory effects on the five classes of cytochrome P450 enzymes, namely CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The results obtained in this study have confirmed the suppression of CYP1A2 by Pioglitazone, Englitazone and Ralitoline, CYP2C19 inhibition by all the other ligands except Etozoline and Teneligliptin, CYP2C9 inhibition by all the other ligands except

Ralitoline, Etozoline and Tenegliptin, CYP2D6 inhibition by all the other ligands except

Troglitazone, Balaglitazone, Ralitoline, Etozoline and Ciglitazone and CYP3A4 inhibition by

all other ligands except Ralitoline and Etozoline.

Table 4.3: ADMET properties of Thiazolidinedione based drugs.

S. r. N o.	Ligands	Lipophi licity (Conse nsus Log $P_{o/w}$)	BBB Perm eant	P-gp subst rate	CYP 1A2 Inhib itor	CYP2 C19 Inhibi tor	CYP 2C9 Inhib itor	CYP 2D6 Inhib itor	CYP 3A4 Inhib itor	Log K_p (skin permea tion)
1.	Pioglitaz one	3.09	No	No	Yes	Yes	Yes	Yes	Yes	-5.81cm/ s
2.	Englitaz one	3.52	No	No	Yes	Yes	Yes	Yes	Yes	-5.30cm/ s
3.	Troglita zone	4.12	No	Yes	No	Yes	Yes	No	Yes	-5.39cm/ s
4.	Balaglit azone	2.37	No	No	No	Yes	Yes	No	Yes	-6.79cm/ s
5.	Ralitolin e	2.15	Yes	No	Yes	Yes	No	No	No	-6.53cm/

									s
6.	Etozoline	1.44	No	No	No	No	No	No	No	-6.88cm/s
7.	Lobeglitazone	3.13	No	No	No	Yes	Yes	Yes	Yes	-6.18cm/s
8.	Rosiglitazone	2.36	No	No	No	Yes	Yes	Yes	Yes	-6.27cm/s
9.	Ciglitazone	3.62	No	No	No	Yes	Yes	No	Yes	-4.98cm/s
10	Teneligliptin	1.76	No	No	No	No	No	Yes	Yes	-7.19cm/s

4.4. Bioactivity score prediction

The analysis of bioactivity scores indicated that none of the Thiazolidinedione-based drugs exhibited complete activity, as outlined in the materials and methods section. Troglitazone had high activity as a ligand for G protein-coupled receptors (GPCRs) and nuclear receptors, as well as an inhibitor for proteases and enzymes. It demonstrated moderate activity as a modulator for ion channels and an inhibitor for kinases. Englitazone exhibited activity as a ligand for G protein-coupled receptors (GPCRs), a modulator of ion channels, an inhibitor of kinases and proteases, while demonstrating moderate activity as a ligand for nuclear receptors

and an inhibitor of enzymes. Teneligliptin demonstrated considerable activity, as all of its readings exceeded the predetermined threshold levels. Table 4.4 provides the information pertaining to the prediction of bioactivity scores for the Thiazolidinedione based drugs.

The current study's findings indicated that Englitazone, Troglitazone and Teneligliptin Thiazolidine based ligands are biologically active molecules than the rest of the compounds as their bioactivity scores are not greater than -0.50. Thus, after interacting with GPCR ligands, nuclear receptor ligands, or acting as inhibitors of proteases and other enzymes, these ligands are capable of exerting physiological responses via different methods.

Table 4.4: Bioactivity score prediction of Thiazolidinedione based drugs.

Sr. No.	Ligands	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
1.	Pioglitazone	0.25	-0.51	-0.71	0.64	-0.09	0.05
2.	Englitazone	0.15	-0.65	-0.90	0.50	-0.14	0.00
3.	Troglitazone	0.22	-0.48	-0.79	0.74	0.05	0.11
4.	Balaglitazone	0.04	-0.75	-0.74	0.12	-0.43	-0.16
5.	Ralitoline	-0.58	-0.55	-0.69	-1.15	-0.99	-0.62

6.	Etozoline	-0.41	-0.55	-0.72	-0.62	-0.35	-0.38
7.	Lobeglitazone	0.12	-0.40	-0.32	0.20	-0.23	-0.01
8.	Rosiglitazone	0.15	-0.65	-0.61	0.35	-0.21	-0.07
9.	Ciglitazone	-0.03	-0.80	-1.04	0.54	-0.25	-0.06
10.	Tenegliptin	0.35	0.18	0.03	-0.40	0.72	-0.10

4.5. Druglikeness and Toxicity potential study

Assessment of drug-likeness and toxicity potential of selected Thiazolidinedione based drugs revealed that all the ligands are non-mutagenic while Etozoline possesses low tumorigenic effects. Ralitoline and Etozoline were found to have high toxic effects while Ciglitazone has low toxic effects on the reproductive system and one of the ligands, Ralitoline, was found to be an irritant. A positive druglikeness evaluation result for all the ligands except Etozoline and Ciglitazone suggested that this molecule primarily contains components found in commercial drugs. Pioglitazone, Englitazone, Troglitazone, Balaglitazone, Ralitoline, Lobeglitazone, Rosiglitazone and Tenegliptin have depicted positive scores for drug-likeness. The details of drug-likeness and toxicity assessment of phytochemicals is given in Table 4.5 and 2D and 3D visual representation is given in Figure 4.5.

Table 4.5: Druglikeness and Toxicity potential assessment of Thiazolidinedione based drugs.

Sr. No.	Ligands Name	Druglikeness	Mutagenic	Tumurogenic	Reproductive Effective	Irritant
1.	Pioglitazone	3.9631	None	None	None	None
2.	Englitazone	3.6632	None	None	None	None
3.	Troglitazone	4.0512	None	None	None	None
4.	Balaglitazone	6.1075	None	None	None	None

5.	Ralitoline	2.1607	None	None	High	High
6.	Etozoline	-2.1235	None	Low	High	None
7.	Lobeglitazone	7.5278	None	None	None	None
8.	Rosiglitazone	7.5038	None	None	None	None
9.	Ciglitazone	-0.30988	None	None	Low	None
10.	Teneligliptin	9.67	None	None	None	None

(a)   (b)
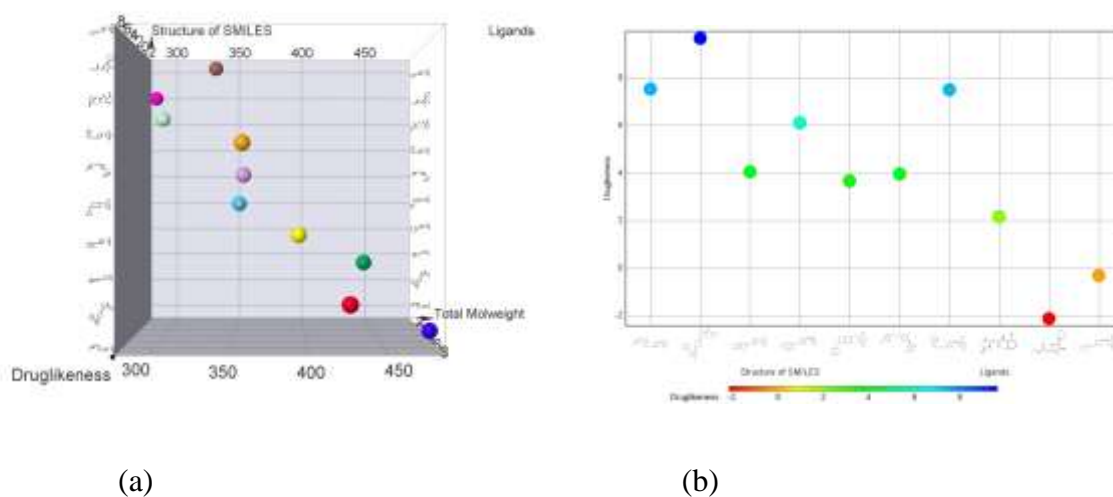
Figure 4.5: Druglikeness of Thiazolidinedione based drugs   (a) 3-D point plot   (b) Scatter plot

4.6. Molecular Dynamics Simulations

The structural flexibility of Heamagglutnin-Neuraminidase (HN) – Troglitazone, Heamagglutnin-Neuraminidase (HN) – Englitazone, Heamagglutnin-Neuraminidase (HN) – Teneligliptin and Matrix (M) – Rosiglitazone was evaluated by performing MD simulations. The complexes were chosen based on binding affinity, non-mutagenic, non-tumorigenic behavior, and protease inhibitory potential of Troglitazone and Teneligliptin and enzyme

inhibitory potential of Englitazone. The findings of our study revealed that the root mean square fluctuation (RMSF) value of Heamagglutnin-Neuraminidase (HN) was initially observed to be 8 Å. However, this value decreased during interaction with Troglitazone and Englitazone, resulting in a recorded RMSF value range of 0.2 Å - 4.0 Å for the HN-complex. In a similar vein, the root mean square fluctuation (RMSF) of the Matrix (M) protein exhibited a decrease (4.5 Å) and shown reduced fluctuations subsequent to its interaction with Rosiglitazone. The selected complexes were deemed to have stable interactions due to their RMSF values, which were found to be in close proximity to the optimum value of 3.8 Å.
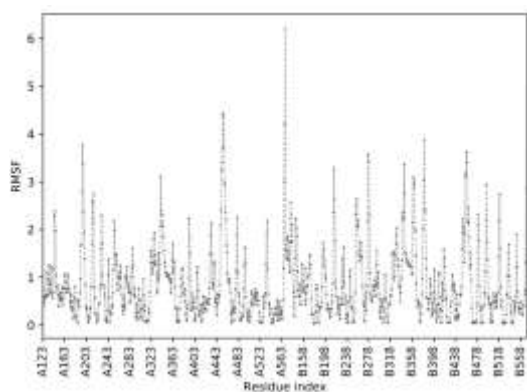
a) HN Protein
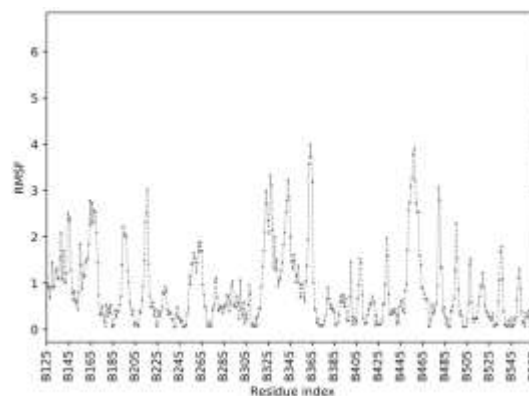
b) HN Protein with Troglitazone
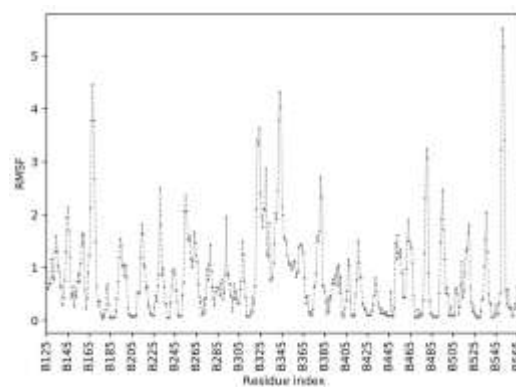
c) HN Protein with Englitazone

Figure 4.6: Representation of value of root mean square fluctuation (RMSF) of a) Haemagglutinin-neuraminidase (HN) protein; b) RMSF plot of HN protein with Troglitazone; c) RMSF plot of HN protein with Englitazone.

a) Nucleocapsid Protein
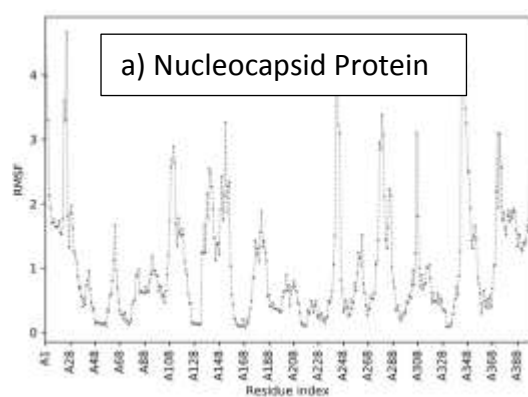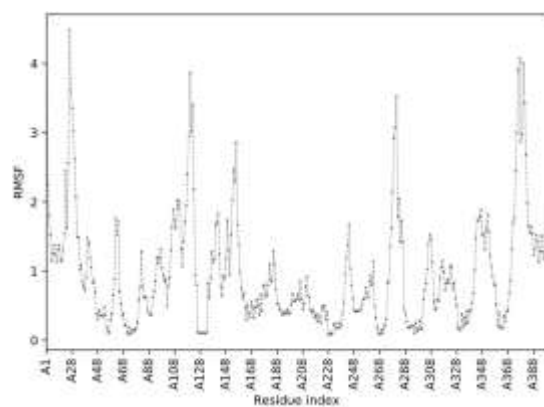
b) Nucleocapsid Protein with Troglitazone

Figure 4.7: Representation of value of root mean square fluctuation (RMSF) of a) Nucleocapsid (N) protein; b) RMSF plot of N protein with Troglitazone.
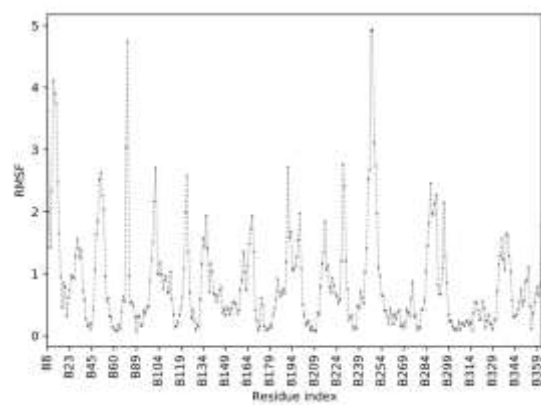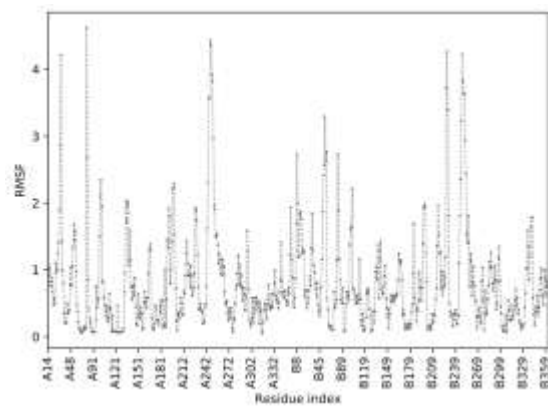
a) Matrix Protein

b) Matrix Protein with Rosiglitazone
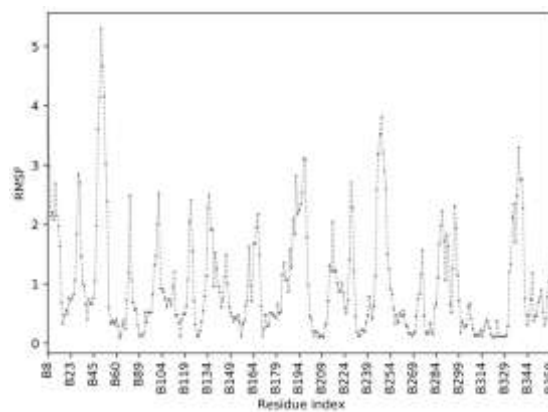
c) Matrix Protein with Ciglitazone

Figure 4.8: Representation of value of root mean square fluctuation (RMSF) of a) Matrix (M) protein; b) RMSF plot of M protein with Rosiglitazone; c) RMSF plot of M protein with Ciglitazone.
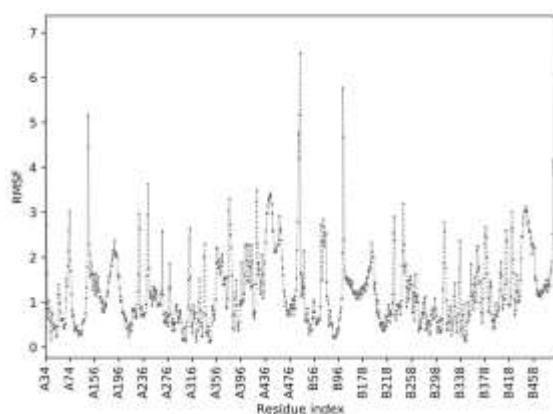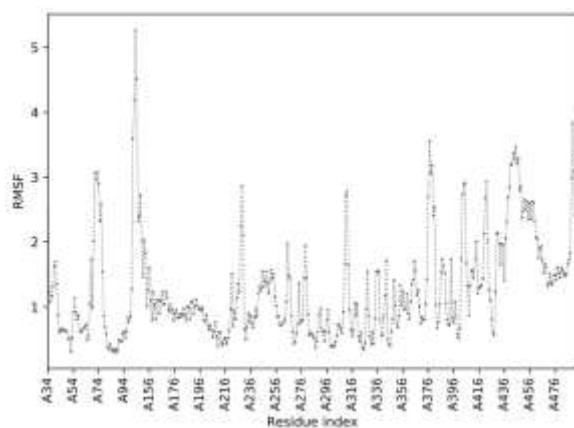
a) Fusion Protein

b) Fusion protein with Balaglitazone

Figure 4.9: Representation of value of root mean square fluctuation (RMSF) of a) Fusion (F) protein; b) RMSF plot of F protein with Balaglitazone.

Discussion

The virus known as Newcastle Disease Virus (NDV) is classified as an enveloped virus, characterized by a single stranded negative sense RNA genome. NDV is a member of the Avulavirus genus within the Paramyxoviridae family. The virus was first detected in 1926 in Newcastle, United Kingdom, and has since had profound implications for the worldwide poultry sector (Dortmans et al., 2011). Since, there have been no effective therapeutics available against NDV and mass vaccination has not been effective worldwide because of continuously evolving antigenic nature of the virus the NDV spread has been on the rise globally. The poultry sector is one of Pakistan's largest and the country continues to suffer enormous economic losses as a result of the frequent outbreaks in various parts of the country (K. M. Dimitrov et al., 2017).

Some of the currently reported antiviral agents and extracts in experimental phase have shown great results which includes extracted sulphated polysaccharides from *Ulva clathrate* and *Cladosiphon okamuranus* Seaweeds (Aguilar-Briseño et al., 2015), *Azadirachta india* (Neem) Bark extract, Nigella Sativa Extract and an antiparasitic drug which is based on member of Thiazolide family called Nitazoxanide (Antony et al., 2020). But designing a novel drug out of these extracts and antiviral agents is an expensive and time-consuming process. From target identification to marketing approval, novel drugs typically take over 12 years also not to forget the extreme durations of experimental phases and successful clinical trials for a drug to be manufactured according to the exact standards. So, prior to the experimental phase, in silico studies can save significant time and money.

The strategy of repurposing drugs used in this study is proved to be successful in finding targeted antiviral therapeutics. Hence, predicting drug similarity, oral bioavailability, efficacy, toxicity risk assessment (Siddiqui et al., 2022), and so on has been helpful in

assessing the potential of selected Thiazolidinedione based drugs prior to wet-lab testing using a planned computational strategy for rational drug design. It is more efficient and economical to conduct pharmacodynamic and pharmacokinetic screening and prediction of phytoconstituents in a computer model.

The objective of the present investigation was to investigate the potential inhibitory effects of drugs based on Thiazolidinedione, which had already been marketed as anti-diabetic drugs, against four targeted structural proteins of NDV employing computational methods (including molecular and cheminformatics technologies). These drugs have also been reported as anti-viral, anti-inflammatory, cytotoxic, anti-microbial, anti-fungal, anti-tumor and anti-cancer (Sahiba et al., 2020).

The present study investigates the binding affinities of ten Thiazolidinedione-based drugs against four specific structural proteins of the Newcastle Disease Virus, namely Heamagglutinin-Neuraminidase protein (HN), Fusion protein (F), Matrix protein (M), and Nucleocapsid protein (NC). This investigation employs molecular, pharmacokinetic, and in-silico methodologies. The HN protein assumes a crucial function in the primary attachment of the virus to host cells through its interaction with sialic acid receptors located on the cell membrane. The enzymatic activity of neuraminidase facilitates the liberation of viral offspring from host cells by the hydrolysis of sialic acid residues. The F protein undergoes a structural alteration that is initiated by the HN protein, hence facilitating the viral entry into the host cell. The fusion process facilitates the liberation of the viral DNA within the host cell, thereby commencing the process of viral replication. The M protein is of utmost importance in facilitating the assembly and budding process of recently generated NDV particles. The protein plays a crucial role in the development of viral filamentous structures and is essential for the effective release of viral particles. It interacts with the viral nucleocapsid (containing the viral RNA genome) and the inner layer of the viral envelope.

The NP protein encapsidates the viral RNA genome, forming the helical ribonucleoprotein (RNP) complex. It is involved in viral RNA replication, transcription, and packaging into new viral particles. The NP protein is also critical for viral assembly and is a target for host immune responses (Ganar et al., 2014). Deeper knowledge of the structure and expanded role of these NDV structural proteins will aid in speeding up the development of antiviral drugs targeting specifically HN and F proteins.

In recent years, there has been notable progress in the field of in silico approaches, including molecular docking, pharmacology networks, and molecular dynamic simulations. These advancements have made substantial contributions to the identification and categorization of possible pharmaceutical agents. Within the field of computational drug discovery and development, molecular docking stands out as a notable technique utilized to forecast the spatial arrangement of tiny molecules that are connected to a binding pocket of an enzyme or receptor. The findings from the molecular docking research conducted using AutoDockVina indicate that Troglitazone exhibits the highest binding affinity with the Heamaglutinin-Neuraminidase (HN) protein of the Newcastle Disease Virus, with a binding energy (BE) of -9.2 kcal/mol. Following Troglitazone, Englitazone has the second highest binding affinity with a BE of -9.1 kcal/mol, while Balaglitazone exhibits a slightly lower binding affinity with a BE of -8.9 kcal/mol. Balaglitazone and Lobeglitazone have demonstrated the highest binding affinities (-7.3 kcal/mol) in the event of a binding interaction with the Fusion protein (F) of NDV. The binding energies of the compounds Rosiglitazone, Balaglitazone, Pioglitazone, and Ciglitazone are -8.4 kcal/mol, -8.3 kcal/mol, -8.1 kcal/mol, and -8.1 kcal/mol, respectively, have shown best binding affinities against Matrix Protein (M). While in case of Nucleocapsid protein (NP), **Troglitazone** (BE = -7.8 kcal/mol) followed by **Balaglitazone** (BE = -7.5 kcal/mol) have shown the best binding affinities as analyzed by AutoDockVina. On the basis of these binding affinities, Troglitazone, Englitazone,

Rosiglitazone and Ciglitazone have been found to be the most effective Thiazolidinedione based drugs against Newcastle Disease Virus.

For this study, ten Thiazolidinedione based drugs were selected based on their potential as antiviral drug, such as anti-HIV, and tested using Lipinski's rule of five which predicts high absorption/permeability when MW < 500, clog P # 5.0, and there are ≤5 H-bond donors and ≤10 H-bond acceptors, and no more than 1 Lipinski's violation is tolerated to ensure maximum bioavailability. Out of ten Thiazolidinedione based drugs, all of them met the requirements for a good lead compound (Table 4.2). The Thiazolidinedione based drugs were further analyzed for their drug-likeness potential and before in vitro and in vivo testing could commence, toxicological and bioavailability data were also gathered to prevent potentially harmful substances for future drug screening.

All of the selected Thiazolidinedione based drugs showed high lipophilicity indicating good solubility but most of them were unable to cross blood-brain barrier except **Ralitoline**. Only **Troglitazone** was found positive as permeable glycoprotein substrate (P-gp substrate) while rest of the drugs showed negative results. Kp refers to the rate at which a chemical can pass through the epidermis, the outermost layer of skin. In a surprising turn of events, all ten Thiazolidinedione based drugs showed negative Kp values, indicating that they are less likely to be absorbed topically (Table 5). Toxicity risk assessment suggests that Pioglitazone, Englitazone, Troglitazone, Balaglitazone, Lobeglitazone, Rosiglitazone, and Teneligliptin are expected to be harmless because they did not show any mutagenic, tumorogenic, irritating, or reproductively hazardous properties.

Molecular dynamics (MD) simulations are helpful tools for learning about the multiscale dynamics of biological macromolecules. The RMSD is always positive, and a perfect fit to the data would have an RMSD of 0, which is impossible to achieve in practice. In most cases,

a smaller RMSD indicates a more accurate model when compared to the true structure. The root-mean-squared deviation (RMSD) during a time period can be thought of as the RMSF when a dynamic system oscillates around a well-defined average location. MD Simulations validated the structural flexibility of the HN/Troglitazone docking complex, HN/Englitzone docking complex, M/Rosiglitazone docking complex, M/Ciglitazone docking complex, NP/Troglitzone docking complex and F/Balaglitazone docking complex as their root mean square value varied below 5Å. RMSF values are used to compute each residue's atomic positional variation based on its C-alpha (Ca) atom. In the present study, the high affinity complexes HN/Troglitazone and F/Balaglitazone of NDV complex displayed a very low deviation of 3.8-4.0Å and 4.9-5.1Å respectively compared to the original targeted protein which displayed a deviation of 6.1Å and 6.5Å respectively.

The current investigation used a number of prediction methods to speculate on the oral bioavailability of drugs; this could pave the way for the creation of new, safer drugs. Before moving to the experimental stage, in silico investigations can save a significant amount of money and time. As these Thiazolidinedione based drugs have been shown to be bioavailable, druglike, and largely devoid of harmful and mutagenesis consequences, they can be investigated further in vitro and in vivo as possible antiviral drugs for the treatment of Newcastle Disease Virus.

Conclusion & Future Prospects

To conclude, Newcastle Disease, a highly pathogenic condition with significant implications for the poultry industry, has been a subject of extensive investigation for a considerable period of time. Upon reflection, previous endeavors to lessen the impact of this disease have predominantly focused on measures related to immunization and the implementation of management practices. Nevertheless, the ongoing progression of the disease, in conjunction with the complexities surrounding the effectiveness of vaccinations and the fluctuating levels of virulence, necessitates the development of inventive remedies.

This study has utilized a range of prediction methodologies to assess the oral bioavailability of diverse substances. The utilization of these methodologies has shed light on several encouraging repurposed pharmaceuticals that exhibit prospective effectiveness against four crucial targeted structural proteins of Newcastle Disease Virus (NDV). The utilization of in silico research has considerable potential in optimizing the drug discovery process, presenting notable advantages in terms of resource allocation and time efficiency prior to embarking on the experimental stage.

A comprehensive investigation of 10 Thiazolidinedione-based compounds, including Troglitazone, Englitzone, Rosiglitazone, Ciglitazone, and Balaglitazone, has revealed a distinct and significant enhancement in their efficacy against the intended protein targets. This enhances the potential for Thiazolidinedione-based medicines to be considered as viable candidates for medication development. As we contemplate the future, these chemicals are positioned for thorough examination in laboratory settings and living organisms, tracing their potential as antiviral agents in the ongoing struggle of the chicken industry against Newcastle Disease. The potential utility of these compounds is underscored by their shown bioavailability, drug-likeness, and apparent lack of toxic and mutagenesis consequences.

This study is currently at a critical point, when computational discoveries present a novel direction in the pursuit of efficient therapies against NDV. By providing clarification on prospective drugs that selectively bind to crucial viral proteins, we are making progress in closing the divide between computational forecasting and real-world implementation. This study not only provides opportunities for additional experimental verification but also emphasizes the importance of interdisciplinary partnerships, including computational methods, virology, and veterinary knowledge.

In summary, the future presents an opportunity for additional investigation in the field, wherein these reutilized medications, supported by computational evidence, have the potential to serve as fundamental components in the fight against Newcastle Disease. This work serves as a leading force in the field of computational antiviral research, aiming to imagine a future in which the theoretical potential of these chemicals materializes into significant advancements. These advancements would not only protect the well-being of poultry but also contribute to the long-term viability and expansion of the industry.

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