MOLECULAR DYNAMICS SIMULATIONS OF SMALL MOLECULE DRUGS FOR DYSFERLINOPATHY TARGETING LYS85 AND GLU64 IN DYSFERLIN (DYSF) PROTEIN

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Abstract
Background: Dysferlin is a Ca^{2+}-activated lipid-binding protein. Dysferlin has a crucial role in the regulation of the immune system and the acceleration of muscle repair through the process of muscle regeneration. Muscular dystrophy emerges in dysferlin-deficient muscle due to defective membrane repair and significant muscular inflammation.
Methods: This study proposes an in-silico approach employing molecular docking to suggest potential small molecules for the therapeutic intervention of muscular dystrophy (MD) by inhibiting mutated dysferlin. The extensive information was gathered through a vast literature to be processed for the molecular docking of dysferlin i.e. DYSF and the screened small molecule drugs from ZINC database. The compounds that adhere to the Lipinski rule of an ideal medicine were chosen, and a docking simulation was performed using the Patchdock server.
Results: The molecular docking and molecular dynamics simulations of dysferlin protein with ZINC98607668 and ZINC98606149 drug complexes yielded remarkable results, revealing a substantial correlation with the dysferlin protein's amino acid residues LYS85 and GLU64. These compounds have affinities towards the dysferlin target site, rendering them suitable pharmaceutical agents for treating dysferlinopathy, particularly in case of LGMD2B.
Conclusions: In concern of drugs screening and application for muscular dystrophy it is suggested that additional research must be done to conduct advanced sources on various drug-ligands involved from the recognized databases to identify the most lethal configuration for dysferlinopathy engagements in genetic behavior.

Keywords: LGMD2B, Dysferlin, Dysferlinopathy, Molecular docking, Simulation, Muscular dystrophy

1. Introduction

Muscular dystrophy, a broad term for a collection of genetic disorders, develops as a weakening condition that specifically targets the musculature, leading to a steady decline in strength and the degeneration of affected muscles. Clinically, this disorder is distinguished by a gradual weakening and subsequent wasting of the muscles that become vulnerable to its insidious effects. The category

of inherited muscular disorders encompasses a range of debilitating conditions [1], namely Emery-Dreifuss muscular dystrophy, Becker muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD), Duchenne muscular dystrophy (DMD), Fukuyama/non-Fukuyama congenital muscular dystrophy, and distal muscular dystrophy involving Miyoshi myopathy (MM) [2]. These disorders, characterized by their genetic basis, manifest in different forms, leading to progressive muscle weakness and functional impairment. Including these disorders within this category highlights the shared underlying mechanisms and clinical features that unite them while also acknowledging the distinct nuances and variations among them. [3] Limb-girdle muscular dystrophy (LGMD) is a complex condition with progressive and symmetrical development of muscle weakness and atrophy. This lethal disorder primarily targets the proximal muscles crucial for everyday movements and activities [4]. As the name suggests, LGMD affects the muscles surrounding the limbs and girdle, leading to mobility and physical function difficulties. One of the remarkable aspects of LGMD is its gradual nature [5], as the symptoms tend to develop slowly over time. This slow progression allows individuals impacted by LGMD to adapt and cope with the changes in their muscle strength and function [6,7]. The inheritance pattern of LGMD2B [8] is characterized by a recessive mode [9], which may be explained by mutations occurring in the dysferlin (DYSF) gene.

The genetic modification leads to a gradual decline and decrease in strength in the muscles in the proximal region of the lower limb girdle [10]. However, it is important to note that the effect of LGMD on the proximal muscles can be profound, greatly harming an individual's ability to accomplish essential tasks such as walking, climbing stairs, or even lifting objects [11]. The symmetrical nature of muscle weakness [12] and atrophy in LGMD is another intriguing captivating medical condition that has captured the attention of researchers and clinicians, includes a diverse array of types [13], each with its unique characteristics and implications.

Cellular proteins are integral in controlling and coordinating numerous physiological mechanisms within an organism. These protein molecules are necessary for the proper functioning of different biological processes [14] and impact the development and advancement of diseases. Their influence spans various organism functions [15,16], moving vital cellular processes and maintaining homeostasis. Regulating targeted inherited illnesses, general illnesses, or biological processes [17] in medical research is complex and challenging [18,19]. However, essential advancement has been done in this field through identifying and consuming lead compounds that can modulate the activity of specific target proteins. Identifying lead compounds involves a meticulous and systematic approach, often encompassing various experimental techniques and methodologies. In the field of studies, researchers utilize several methods, including high-throughput screening [20], virtual screening, and structure-based drug design, to identify potential molecules capable of binding with and modulating the functioning of target proteins.

Computer-aided drug design (CADD) is an extensively systematic and advanced approach in drug development [21]. It includes a range of mathematical techniques and algorithms used to identify and evaluate potential drug applicants. By employing target structures, functional properties, and fundamental biological processes, CADD aims to accelerate the discovery and optimization of novel therapeutic agents. At its foundation, CADD involves utilizing advanced computational tools and methodologies to identify and design molecules with the desired pharmacological activity. These tools [22] enable investigators to forecast the communication between potential drug candidates and their target molecules, thereby aiding in the choice of compounds with a high probability of exhibiting therapeutic efficacy. One of the critical aspects of CADD is the use of target structures, which are usually proteins or nucleic acids that serve an essential role in disease processes. Various distinctive design techniques may be implemented based on the information that is easily accessible regarding the receptor structure and how it connects with a ligand. After the lead compounds have been produced, the Lipinski rule [23] of five may be employed to evaluate the drug-like properties of the newly created compounds.

In this study we applied an extensive validation included Molecular Dynamics (MD) simulations [24] to estimating the effects of potential lead compounds. Following the traditional Lipinski's rule of five,

this study can significantly be helpful for future clinical and experimental outcomes to address the genetic issues related to dysferlinopathy and drug designing.

The schema of study presented in (Fig.1) for visual understanding of targeted lead compounds and analysis of Dysferlin protein.
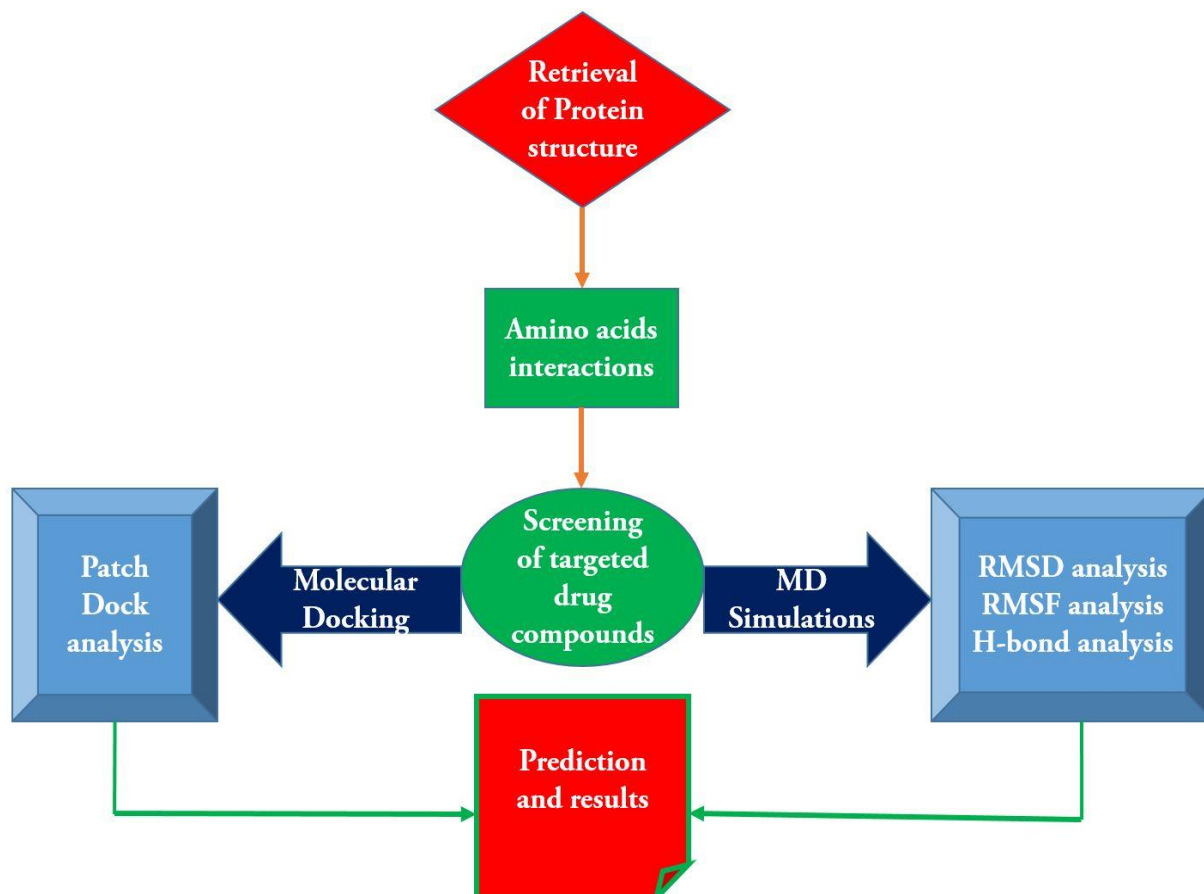

Fig 1: Overall schema of the study presented with potential outcomes using molecular docking and MD simulations approaches

2. Methods

2.1. Retrieval of protein structure

A variation in the DYSF gene leads to the impairment of the dysferlin protein, which usually appears in its natural state. The 3D structure of the Dysferlin protein was accessed from the protein databank [25] https://www.rcsb.org/. This resource enabled the opportunity to obtain the protein structure for subsequent analysis in the current study. Cleaning of protein was performed as the role of water molecules in the process of binding is rare. Therefore, removing water molecules was done to compute and eliminate the potential interference from water molecules in the binding pocket that might impact on the pose search. During the docking process, the objective was to identify molecules that may establish several advantageous contacts with the protein.

For the visualization of protein structure and molecular analysis Discovery Studio [26] v21.1.0.20298 was used.

2.2. Molecular interaction of amino acids and retrieval of drug compounds

The impact of gene variation on the structure and function of proteins exhibits considerable variability, contingent upon the specific protein type and the extent of the variation. The task of accurately predicting the impact of sequence variations on the structure and function of a protein is challenging. To understand protein structure and function comprehensively, it is essential to

consider the target amino acids' precise location and relevance to the protein's function as a whole. Pocket identification in dysferlin is achieved by utilizing CASTp [27], (a computer program freely available). After protein-related data collection, there was a need to select specific database to extract information of the drugs suggesting the involvement for treatment of any muscular dystrophy. Hence, we approached ZINC database [28] for the list of available medications.

2.3. Molecular Docking

After determining the target protein and chemical compound structures retrieval, molecular docking was performed to achieve the chemical molecule's dysferlinopathy inhibitory function. This docking approach interacts with the target protein and chemical substance to generate stable complexes that impact dysferlinopathy-related actions. By precisely placing the chemical molecule in the target protein's binding region, its inhibitory effect may be improved, potentially treating dysferlinopathy. Patch Dock, a web-based molecular docking tool, [29] the program is available at https://bioinfo3d.cs.tau.ac.il/PatchDock/. The protocol of docking was followed by the principle of Quan et al. [29] Computer-based molecular docking anticipated the significant molecules binding orientation and affinity, usually concern with ligands and receptors. In the concept of drug discovery and design depending on its insights on small molecule-target protein interactions. Patch Dock generates docking predictions utilizing shape geometry and electrostatic force interactions with high-end interface. This method transfers the input file using a Protein Data Bank (PDB) [25] format. This format is widely employed in structural biology to store and communicate three-dimensional structures of proteins and nucleic acids. A standardized format PDB contains atomic coordinates, molecular communication, and other information to characterize the complex geometry.

Docked complexes were identified by standard principles, which evaluates the geometric compatibility of the molecules interacting surfaces. Due to their distinctive surface geometries, the lead compounds under the study marked with unique characters. The main phases of these computational procedures are for Molecular Structure Representation, Surface Patch Matching, Filtering, and Scoring [30]. These parameters have had vital impact to the process and providing the precise and dependable results. These sequential methods help out the researchers to identify and describe substances by analyzing molecular structures. The initial stage, molecular structure representation, converts molecular structures into a format that can be processed and compared. This format enables the extraction of necessary features and descriptors for analysis. Following this approach, Surface Patch Matching was employed to locate and align molecular surface updates.

2.4. Molecular Dynamics simulations

The MD simulations setup of top 2 docked drug complexes with Dysferlin protein along native Dysferlin protein were used to prepare by web-based CHARMM-GUI [31] interface having CHARMM36m [32] force field. Nanoscale Molecular Dynamics (NAMD) [33] computer software package used to execute MD simulations for 50 ns on all 3 setups. The protocol for simulations followed by including a cubic box of 15 Å with TIP3P water molecules [34]. Potassium ions were selected in the system to gain charge neutrality. Later, system was submitted to the energy minimization via steepest descent protocols until the energy of system change become less than 1 Kj mol^{-1} and nm^{-1}. In order to keep the adequate structure for simulations a stepwise equilibration of the systems was implemented before trajectories deployment by applying Simulation of system based on constant number (N), constant-volume (V), and constant-temperature (T) (NVT) and An isolated system with fixed pressure (P), a fixed number of atoms (N), and a fixed temperature (T) (NPT) thermostats with Nose-Hoover temperature coupling 300 K and Parrinello- Rahman pressure coupling 1 atm. MD simulations was carried out for all systems at constant pressure of 1 atm and temperature 300 K. Equations of motion were set at time step 2 fs, and coordinates of the systems were saved each 1 ps. Further, electrostatic interactions were calculated by Particle Mesh Ewald (PME) with cutoff of 12 Å. The trajectories were extracted using Visual Molecular Dynamics [35]

(VMD) software package and graphical analysis performed using Python programing language 3.10 version with matplotlib plotting library.

The system used to carry out MD simulations and trajectories analysis having specifications as Graphical Processing Unit (GPU) Intel (R) Core (TM) i9-9900KF CPU @ 3.60 GHz 32.0 GB RAM.

2.5. ADMET properties

Following the completion of the molecular docking and MD simulations processes, it becomes imperative to ascertain the toxicity classification of the primary drug via an assessment of its ADMET [36] characteristics. This step is crucial to deduce conclusions on the selection or rejection of the compound to produce a standard therapy potential for research involved in dysferlinopathy. The ADMET properties are retrieved via SWISSADME [37] (http://www.swissadme.ch) and ProtoxII [38] (https://tox-new.charite.de/protox_II/) servers respectively.

3. Results

3.1. Structural incidences involved in Dysferlin protein

The dysferlin protein's three-dimensional (3D) structure was freely downloaded from the website https://www.rcsb.org/. The systems studied in this study involve the dysferlin crystal structure analysis of the c2a variant 1 (c2av1), the fluid structure of the dysferlin c2a domain in the presence of calcium, the calcium-bound c2a domain obtained from human dysferlin, and the fluid form of the dysferlin c2a domain in the absence of calcium. The X-ray structure of the canonical c2a domain in human dysferlin (4ihb) was subjected to investigation.

This decision was taken due to the lack of prior systems that either exist in a solution state or employ dysferlin isoforms isolated from non-human species. The 3-dimensional structure of the dysferlin protein and drug compounds (ZINC98607668, ZINC98606149) presented in (Fig.2).
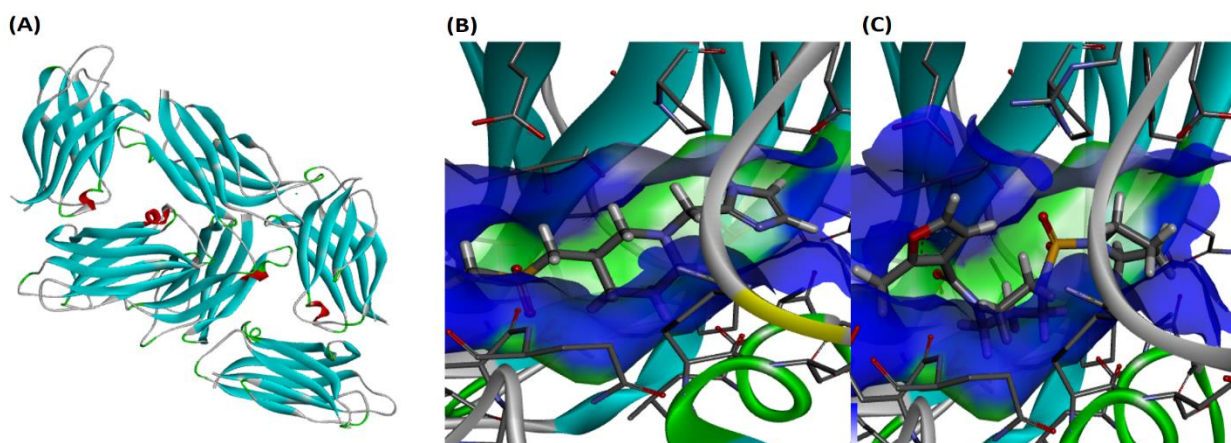

Fig.2. 3D structure of native protein and selected drugs retrieved from the annotated databases presented. (A) X-ray *(4ihb)* illustrates the 3D structure of the dysferlin protein, which consists of six distinct chains marked as A, B, C, D, E, and F. (B) 3D structure of drug compound ZINC98607668 (C) 3D structure of drug compound ZINC98606149 retrieved from ZINC database interacting 4IHB.

Dysferlin, a transmembrane protein with a molecular weight of 230-kDa, resides inside the plasma membrane. The intracellular domains of the entity consist of six C2 domains, which show a much greater size than the small extracellular domain [39]. During the early stages of limb development, particularly at around 5-6 weeks of gestation, dysferlin expression can be observed in skeletal muscle. The C2 domain of this entity has been demonstrated to interact with calcium and phospholipids [40], indicating its involvement in the process of membrane repair.

The prediction of specific amino acids that tend to interact with substances to hinder the mutant variant of dysferlin is necessary. Hence, the identification of pocket atoms inside dysferlin has been

achieved effectively using the CASTp server. Upon identifying the pocket amino acids inside dysferlin, it has become apparent that one specific amino acid should be selectively targeted to inhibit the defective protein linked to dysferlinopathy. Hence, identifying these target atoms limits our focus on screening the compounds interacting with these specific pocket atoms. In the subsequent phase, purifying the target dysferlin protein is imperative to improve its structural conformation for docking. Specifically, it is essential to eliminate any water molecules and ligands associated with the dysferlin protein, hence promoting the stabilization of its structure.

3.2. Molecular docking of dysferlin protein with potential drug compounds

After completing the necessary process to produce a protein appropriate for docking, the next step involves getting the chemical compounds needed for protein-ligand docking.

The ZINC database (https://zinc12.docking.org/) selected chemical compounds following the Lipinski rule of five to accomplish the objective. These substances are further investigated for deeper insights into their interaction with dysferlin. The present research principally examined the chemical compounds listed in Table 1, which selectively relate to the amino acids within the predicted pocket of dysferlin as identified by CASTp. Zinc is a broad database with an extensive array of billions of distinct chemical compounds. Therefore, as discovered in the Zinc database, the Protein Data Bank ID for dysferlin was employed to retrieve a range of compounds with potential therapeutic applications in managing muscular dystrophy. Among the broader array of chemical compounds, a subgroup of 6375 substances has been shown to possess characteristics similar to those found in pharmaceutical molecules. This finding was uncovered when examining the aggregate population data.

Table 1 ADMET Properties analysis of selected drug compounds involved in dysferlinopathy flexibility

Drug Name	Adsorption		Distribution		Metabolism		Excretion	
ZINC98607668	Caco-2 Permeability	-6.078	PPB	14.96%	CYP1A2 inhibitor	0.045	CL	2.306
	MDCK Permeability	5e-06	VD	0.892	CYP1A2 substrate	0.653		
	Pgp-inhibitor	0.008	BBB Penetration	0.515	CYP2C19 inhibitor	0.041		
	Pgp-substrate	0.927	Fu	83.56%	CYP2C19 substrate	0.107	T 1/2	0.718
	HIA	0.043			CYP2C9 inhibitor	0.007		
	F 20%	0.022			CYP2C9 substrate	0.078		
	F 30%	0.416			CYP2D6 inhibitor	0.078		
ZINC98606149	Caco-2 Permeability	-5.354	PPB	82.58%	CYP1A2 inhibitor	0.068	CL	9.593
	MDCK Permeability	1e-05	VD	1.752	CYP1A2 substrate	0.113		
	Pgp-inhibitor	0.004	BBB Penetration	0.85	CYP2C19 inhibitor	0.289		
	Pgp-substrate	0.008	Fu	29.80%	CYP2C19 substrate	0.483	T 1/2	0.29
	HIA	0.006			CYP2C9 inhibitor	0.576		
	F 20%	0.216			CYP2C9 substrate	0.508		
	F 30%	0.089			CYP2D6 inhibitor	0.038		

In contrast, these molecules have distinctive orientations which help their interaction with dysferlin. The compounds obtained from the zinc database have yielded an array of amino acids that dysferlin is expected to interact with, serving as its target molecules. Hence, the findings of this investigation show that only the compounds listed in Table 2 exhibits notable binding interactions with the anticipated amino acid pockets in dysferlin..

Table 2 Potential dysferlinopathy toxicty of LD50 and toxicity class accessed from protox II server

Name	LD50	Toxicity Class
ZINC98607668	500mg/kg	4
ZINC98606149	2450mg/kg	5

The chemical compounds are then subjected to the docking method, yielding outputs targeting dysferlin's Lys85 and Glu64 residues to impede the disease-causing variations. The docking outcomes with interacting amino acids presented in (Fig.3).
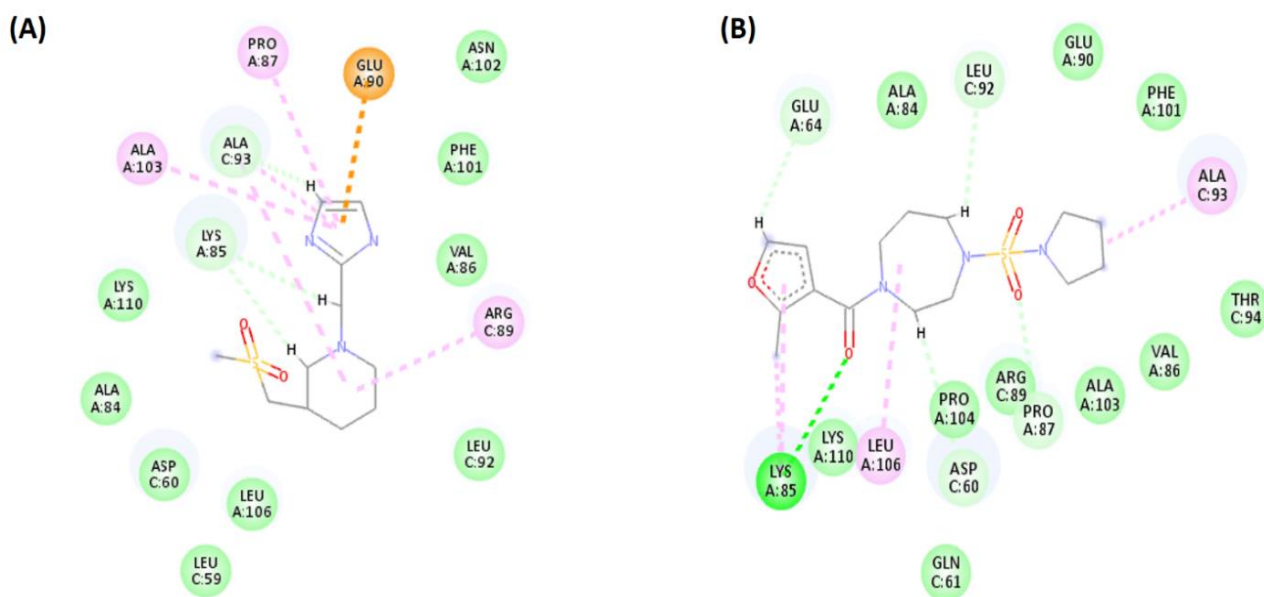

Fig.3. 2D diagram of interacting molecules of Dysferlin protein presented with each drug compound. (A) Interacting amino acid based on bonding power with ZINC98607668 compound (B) Interacting amino acids with ZINC98606149

3.3. Molecular Dynamics Simulations of ZINC98607668 and ZINC98606149 docked complexes
The MD simulations were carried out to understand the stability of docked complexes and also native protein MD simulations performed for the comparative analysis. Root Mean Square Deviation (RMSD) [41], Root Mean Square Fluctuation (RMSF) [42] and Hydrogen bonds analysis were performed for all of the simulations setup. Graphs were generated with respect to 50 ns production relative to the backbone and all protein atoms respectively.

3.3.1. RMSD analysis
In case of native dysferlin protein average RMSD values were found to be 0.6525 ± 0.0953 and steadily increased from 0 to 10 ns then gained equilibration, later from 30 to 50 ns increased and overall RMSD recorded without any fluctuation. For drug ZINC98607668 with dysferlin protein complex average RMSD recorded as 0.6878 ± 0.0915 with increased RMSD from 5 ns and then converged from 15 ns and random fluctuations were observed throughout the simulations. However, in case of ZINC98606149 with dysferlin protein complex average RMSD observed 0.85876 ± 0.07855 and RMSD value gained initially till 04 ns and then converged from 15 to 30 ns with no any significant fluctuations observed thereon.
The detailed graphical representation of each RMSD values at time (s) shown in (figs.4, A-C). There was no lethal distortions observed in all simulations setups for RMSD analysis.

3.3.2. RMSF analysis
Residue-based Root mean Square Fluctuations (RMSF) analysis of 700 total residues in dysferlin protein complexes with ZINC98607668 and ZINC98606149 were determined to understand the flexibility of each residue and details of RMSF shown in graphical representation (figs.4, D-F). The magnitude of fluctuation for each residues calculated by RMSF values and the fluctuation for all of the complexes observed in similar trend with 0.19 to 0.60 for the first 300 residues. Later, low average RMSF was observed from 600 to 700 residues for all of the complexes. This trend in RMSF plot for

complexes indicated binding combination of ZINC98607668 and ZINC98606149 candidate drug
compounds was stable with receptor had no major effect on the flexibility of protein throughout the
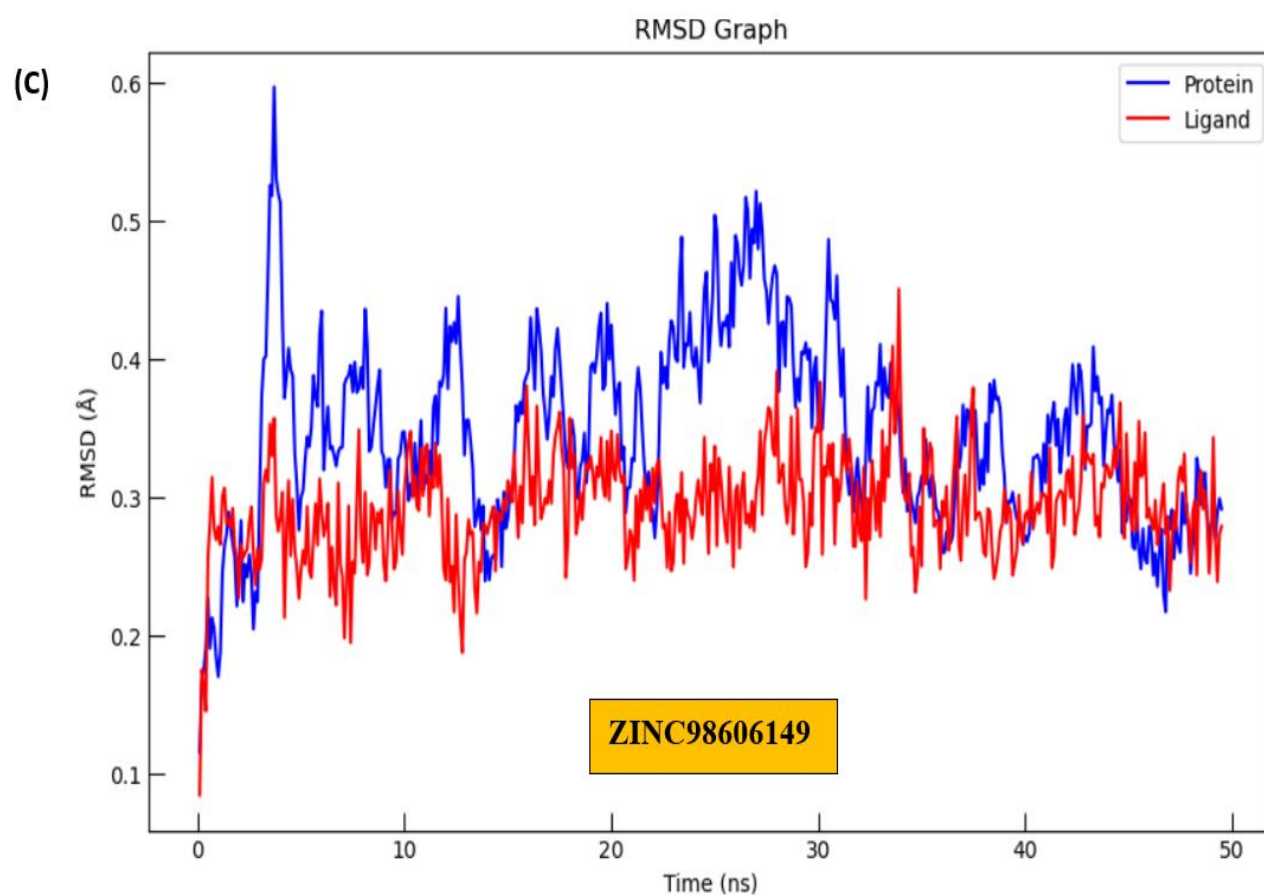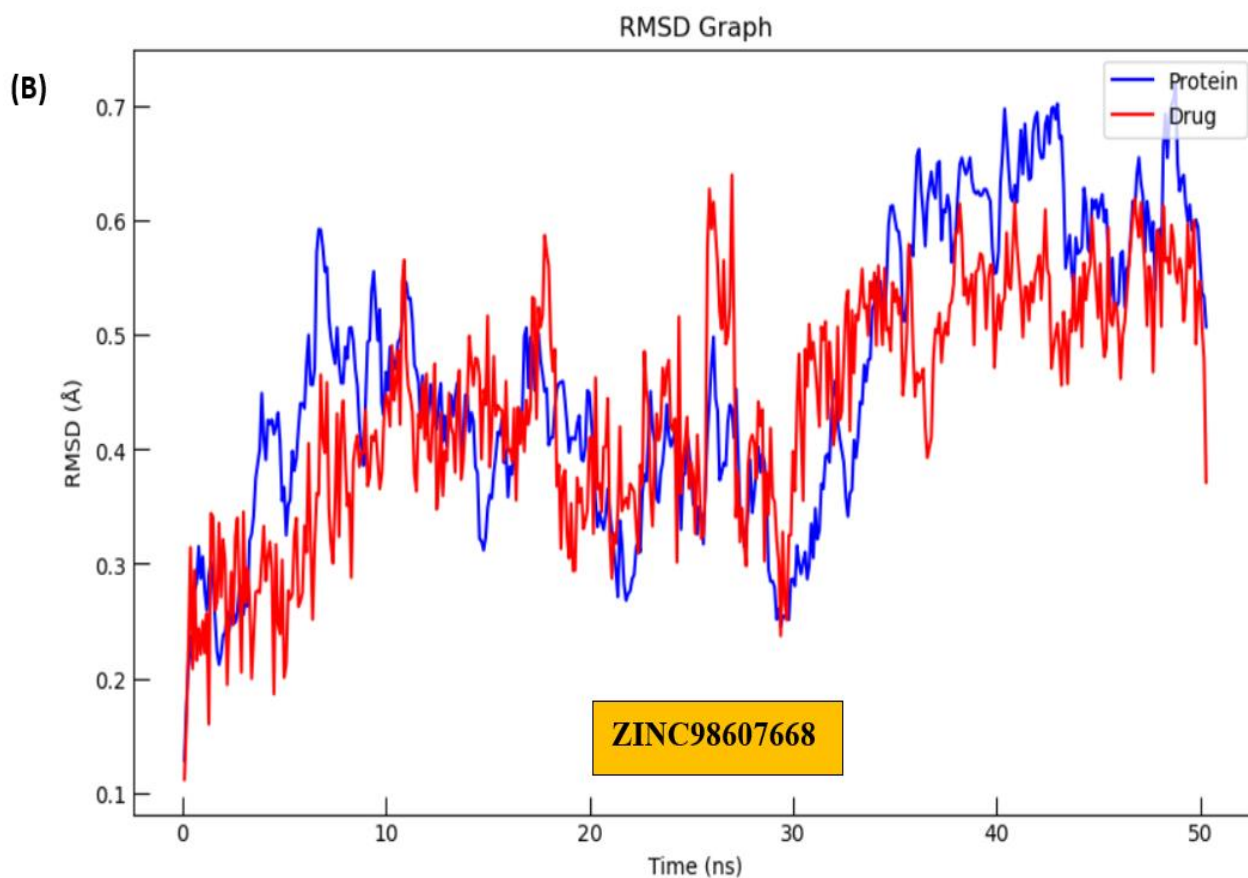simulations.

3.3.3. Hydrogen bond interaction

Hydrogen bond analysis between dysferlin protein and drug compounds was performed and it was
depicted from the graphs that 50 ns MD simulations of the ZINC98607668 drug protein complex
revealed dynamic changes in hydrogen bond formation. Initially, hydrogen bonds began to form at
the 8th nanosecond and persisted continuously until the 29th nanosecond, indicating a period of stable
interactions between the protein and ligand. However, a subsequent phase of inconsistency was
observed, with a cessation of hydrogen bond formation. Interestingly, at the 34th nanosecond, the
formation of hydrogen bonds resumed and persisted until the completion of the MD reaction. This
resurgence suggests a renewed interaction between the protein and ligand, potentially indicating a
shift in the conformational dynamics of the complex.

The analysis of hydrogen bond patterns throughout the simulation underscores the strong interaction
between the protein and ligand, emphasizing the structural stability of the complex over the entire 50-
nanosecond duration of the MD simulation reaction.

Similarly, The 50 ns MD simulations of the ZINC98606149 drug protein complex unveiled
dynamics transformation in hydrogen bond formation, characterized by a relatively low occurrence
of hydrogen bonds. In the initial phase of the simulation, hydrogen bonds started to form at the 11th
nanosecond, suggesting the initiation of interactions between the protein and ligand. This formation
persisted continuously until the 24th nanosecond, indicating a sustained period of stable interaction
between the two molecules. However, in the subsequent phase of the MD simulations, a important
observation emerged that no hydrogen bonds were detected. This absence of hydrogen bond
formation implies a distinct shift in the interaction dynamics between the protein and ligand. The
scarcity of hydrogen bonds during this phase may signify a potential alteration in the conformational
arrangement or the weakening of the binding forces between the two molecules.
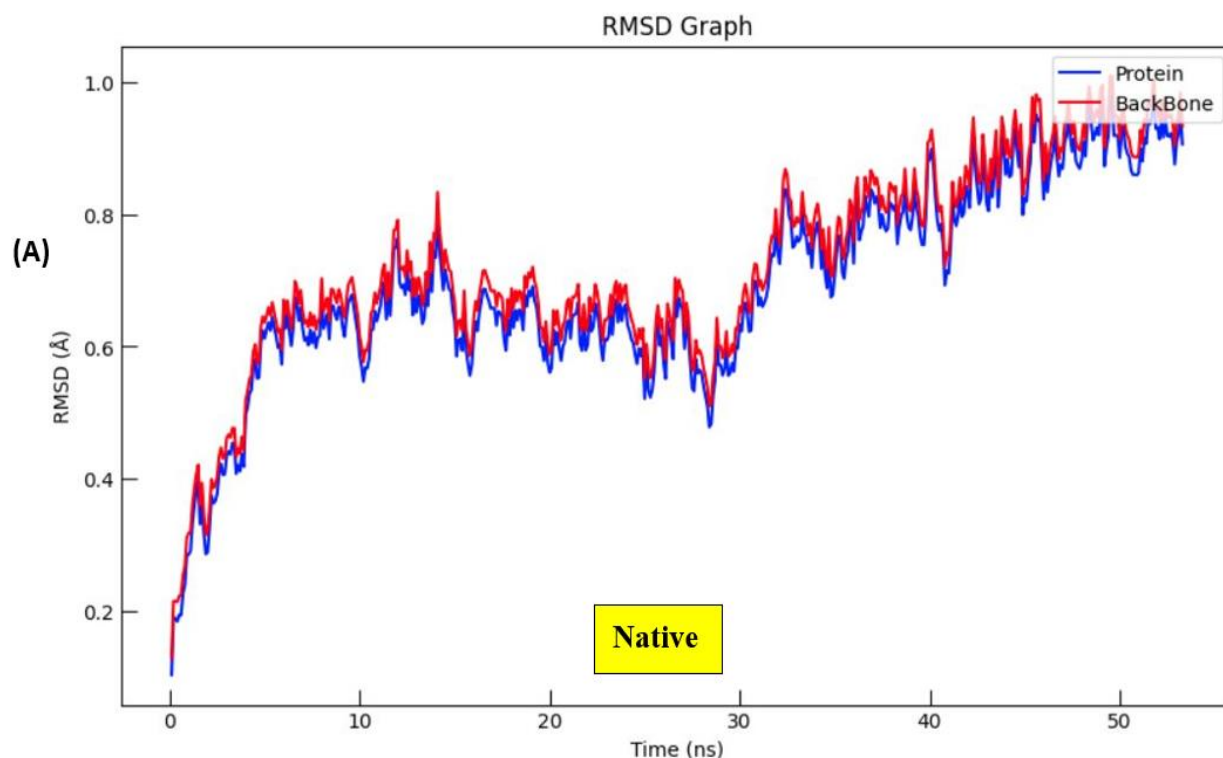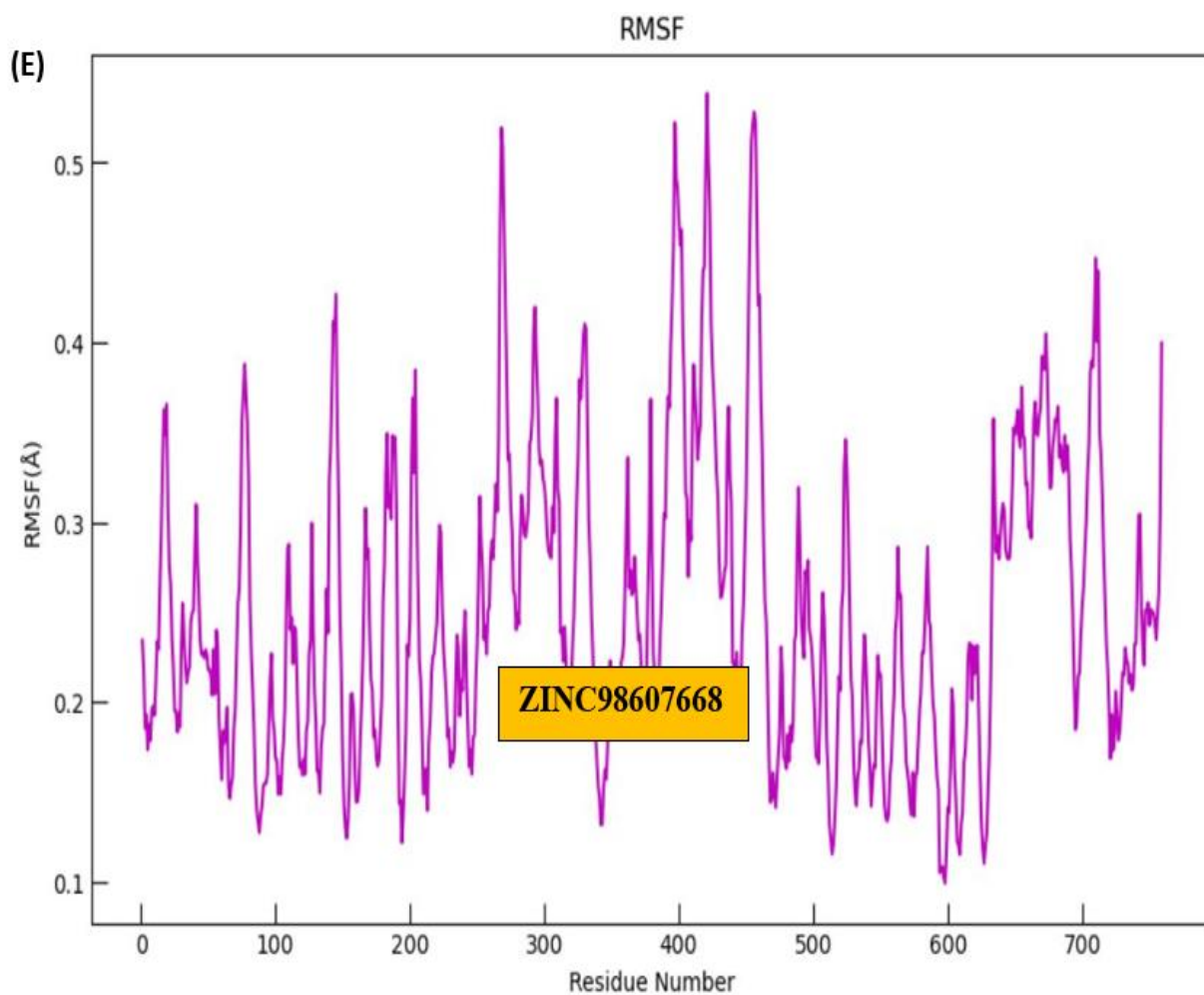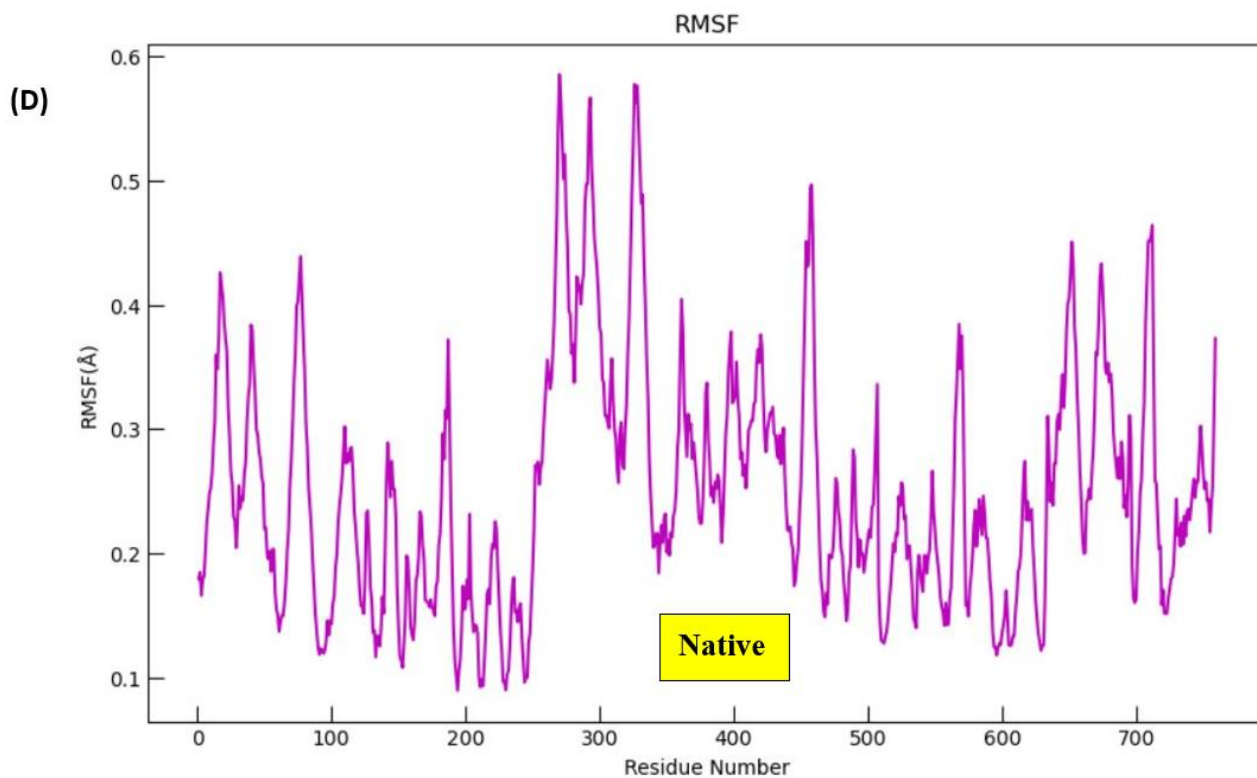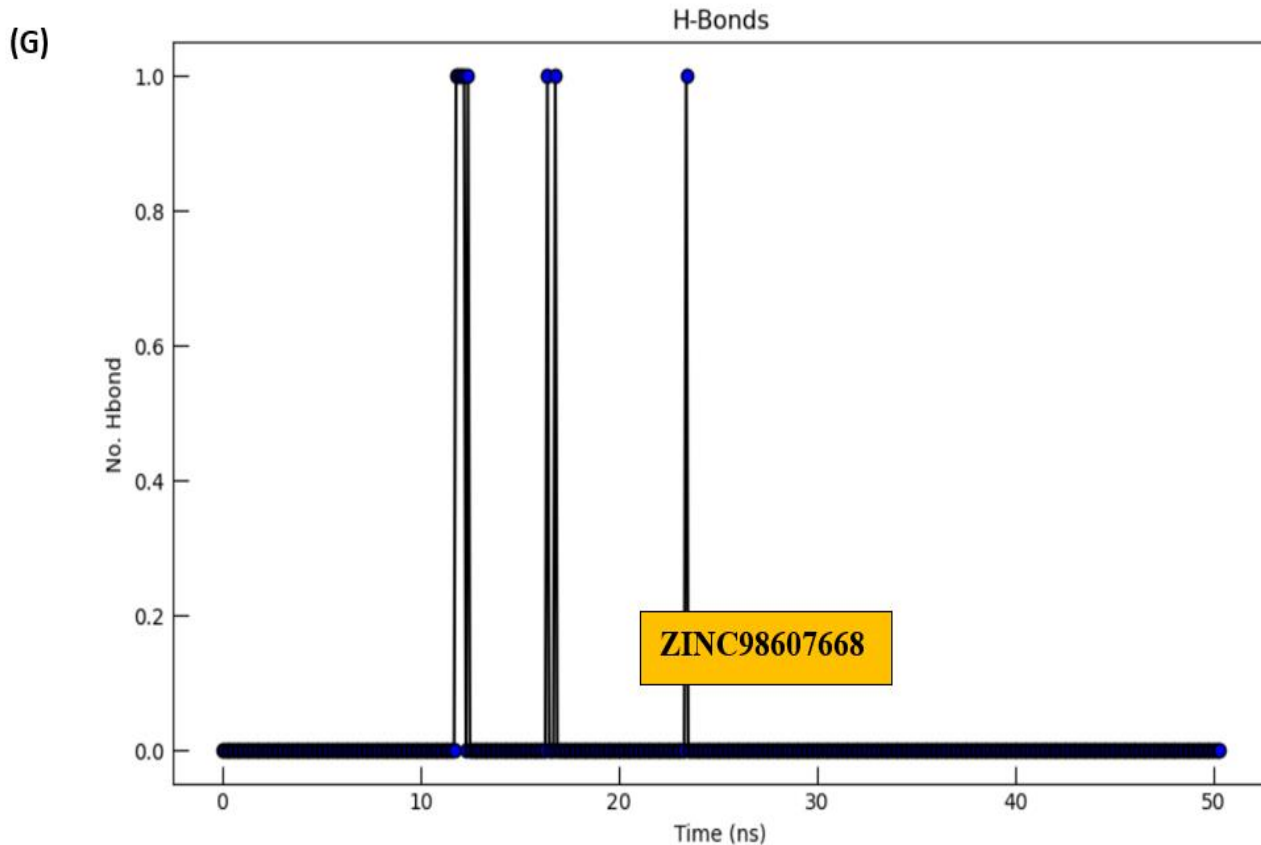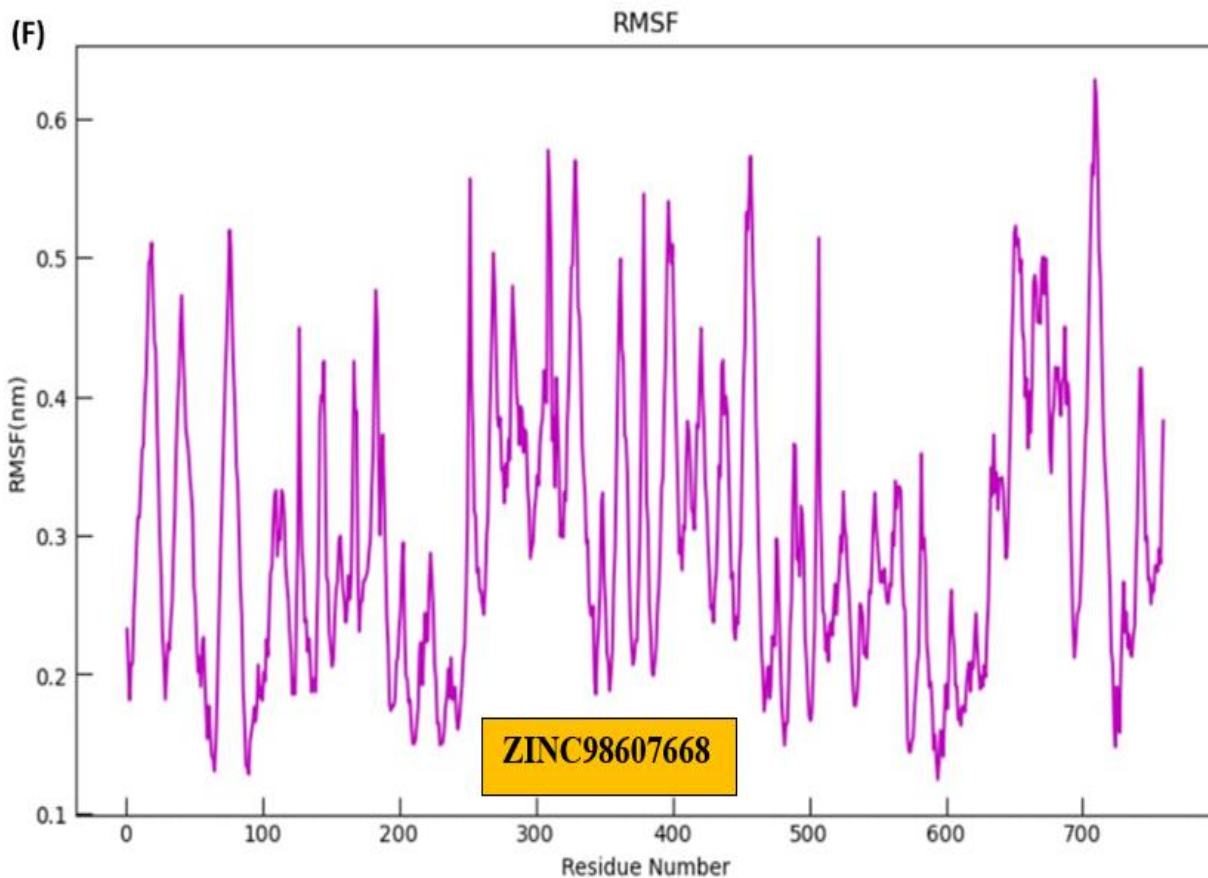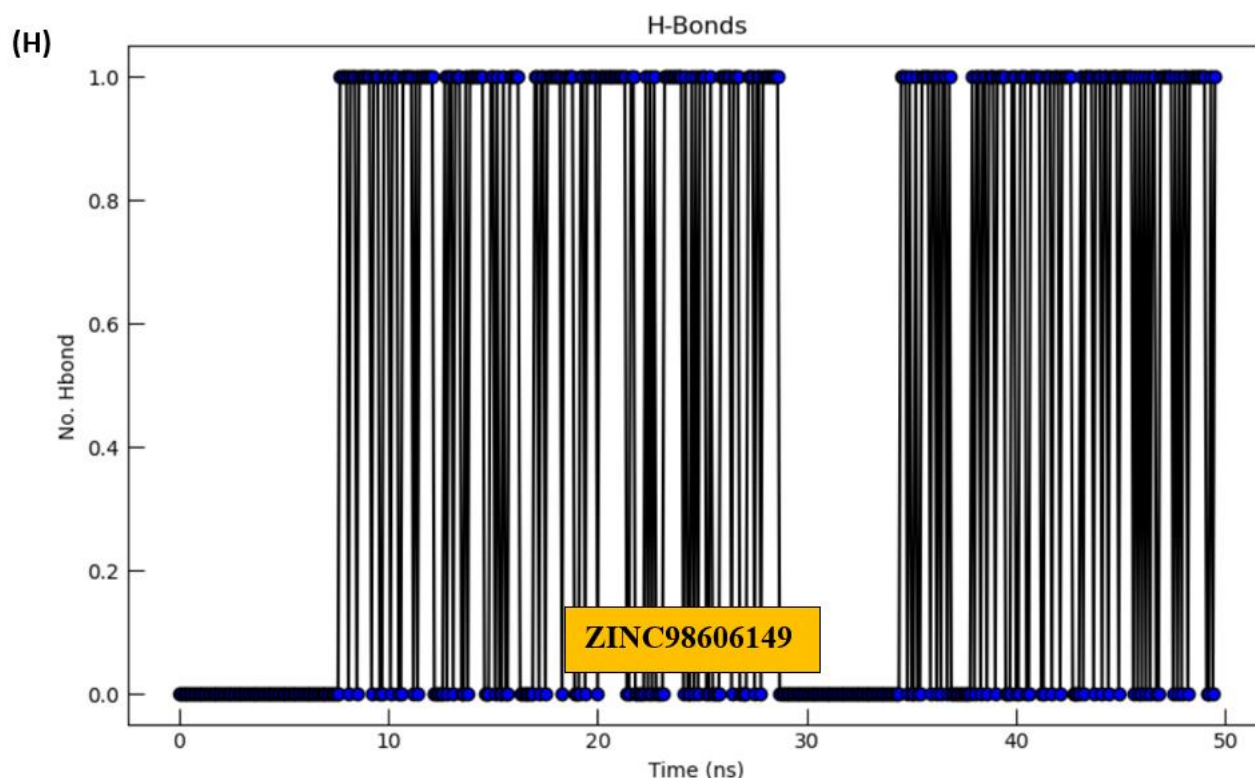
Fig.4. (A) RMSD analysis of native protein structure calculated to determine the stability of protein structure during 50 ns MD simulations (B) RMSD analysis of ZINC98607668 docked complex with dysferlin protein computed to determine the stability of protein structure during 50 ns MD simulations (C) RMSD analysis of ZINC98606149 docked complex with dysferlin protein computed to determine the stability of protein structure during 50 ns MD simulations (D) RMSF analysis of native protein performed to determine the root mean fluctuation for each residues in 50 ns MD simulations (E) RMSF analysis of ZINC98607668 complex performed to determine the root mean fluctuation for each residues in 50 ns MD simulations (F) RMSF analysis of ZINC98606149 complex performed to determine the root mean fluctuation for each residues in 50 ns MD simulations (G) H-bond analysis performed with unique interacting bonds identification for ZINC98607668 protein complex in MD simulations 50 ns (H) H-bonds analysis performed with unique interacting bonds identification for ZINC98606149 protein complex in MD simulations 50 ns.

1.1. ADMET properties analysis

The ADMET Properties comprised on a range of forecast molecular parameters as absorption, distribution, metabolism, excretion, and toxicity. The assessment of ADMET features is a critical factor in determining the toxicity class of the lead compound. This assessment significantly influences the decision-making process around the acceptance or rejection of the mixture for the development of a conventional treatment for dysferlinopathy. The comprehensive ADMET properties analysis of respective drug compounds used in this stidy presented in (Table 1). Since, chemical toxicity evaluation plays a significant role in pharmaceutical research and development. Thus toxicity of the above mentioned chemical compounds was further evaluated by using ProtoxII server [43], in reference to LD50 [44] value and the resulting outcomes are displayed in (Table 2).

The extensive details regarding the absorption, distribution, metabolism, elimination, and toxicity classification of the drugs that have demonstrated promising interactions with the target amino acids in dysferlinopathy. These valuable insights have been obtained using two significant computational tools, by utilizing the capabilities of these advanced platforms, it can be deduced to foresee and assess the pharmacokinetic parameters and toxicological impact of the drugs under current investigation.

Including such information is crucial for improving the knowledge of dysferlinopathy involvement with chemical expression and the potential drug treatments further can be investigated by clinical trials, that may be develop to address this debilitating condition.

4. Discussion

Dysferlinopathy is transmitted via an autosomal recessive pattern. Under the domain of genetic lineage, it is essential to recognize that when an individual suffers from a particular ailment, there is a 25% probability that their sibling is similarly affected. A 50% probability exists for a sibling to develop into an asymptomatic carrier, while a 25% possibility exists for the sibling to stay unaffected and non-carrier of the condition. Once an at-risk sibling is unaffected, their chances of becoming a carrier are 2/3. When specific pathogenic mutations within a family are identified, conducting carrier screenings for at-risk relatives becomes feasible. Additionally, prenatal diagnosis can be made for pregnancies considered to be at high risk. The diagnosis is established via a muscle biopsy and molecular genetic testing. Primary dysferlinopathy is frequently confirmed by Western immunoblotting of muscle specimens.

Dysferlinopathy arises from genetic mutations that develop inside the day's gene, which is thought to encode the dysferlin protein.

In order to find critical and clinical impact of muscular dystrophy number of studies focused on the intriguing LGMD1A, LGMD1B, LGMD1C, and analyzed meticulously linked to dominant loci, further observed to the complexity and fascination surrounding this condition [13]. Moreover, it is noteworthy that several muscular dystrophy disorders, such as LGMD2A, LGMD2B, LGMD2C, and their related variants, are associated with recessive loci in genetic expressions [45]. These genetic loci play a crucial role in the inheritance pattern of these diseases, where both copies of the gene must be affected for the disorder to manifest. This recessive inheritance pattern adds an extra layer of complexity to the understanding and diagnosis of these conditions. By unraveling the intricate genetic mechanisms underlying the chemical pathways, diverse nature of these forms is evident in their etiological heterogeneity, which adds another layer of complexity to their study. Numerous studies highlighted [46] the need for a comprehensive and advanced approach to examining these factors. By recognizing and exploring the various types in considering the aforementioned description, it is worth of noting that esteemed European Neuromuscular Centre, has successfully devised a comprehensive and sophisticated categorization system. This novel system is ingeniously based on the concept of loci, thereby providing an innovative and efficient approach to categorizing various elements within the arena of neuromuscular research.

Currently, no effective treatments are available for individuals who suffer from dysferlinopathy. The fundamental defect in dysferlin insufficiency is the reduced capacity. Therefore, the potential of gene replacement therapy to restore functional dysferlin production and facilitate membrane healing is significant. Nevertheless, the feasibility of implementing dysferlinopathy in real settings remains a distant prospect. According to the latest research on gene-targeted mice, dysferlinopathy may be treated as a therapeutic avenue by emphasizing the complement system [47]. The therapeutic utilization of complement suppression has been widely studied as a potential treatment strategy for various illnesses associated with complement dysregulation [48 49]. Other methods have also being investigated to avoid inflammation as a possible treatment for dysferlinopathy.

The efficacy of anti-inflammatory corticosteroids in dysferlinopathy remains debatable [50 51 52]. The current study highlights the potential of drug therapies, specifically ZINC98607668 and ZINC98606149, as potential methods for treating this condition. Additional research into the signaling pathways involved in regulating inflammatory responses in dysferlinopathy offers promise to improve the development of drugs to reduce the disease [53].

The compound ZINC98607668 exhibits an LD50 value of 500mg/kg, placing it within the toxicity class 4. This classification shows that the combination is thought to have a relatively low level of toxicity. Consequently, ZINC98607668 can be deemed an acceptable candidate for therapeutic intervention in treating dysferlinopathy. The findings presented in Table 4 show that ZINC98607668

exhibits favorable ADME (absorption, distribution, metabolism, and excretion) properties, making it a promising candidate for treating dysferlinopathy. Specifically, the drug demonstrates medium permeability, indicating that it can be effectively absorbed into the bloodstream. This feature ensures that the drug reaches its intended destination and exerts its therapeutic effects. Therefore, based on these ADME properties, ZINC98607668 retains excellent potential as a treatment option for individuals suffering from dysferlinopathy.

Drugs that exhibit a high degree of protein binding may possess a low therapeutic index, suggesting a small margin of safety and efficacy.

The clearance rate of compound ZINC98607668 is reported to be less than 5, indicating a significantly low clearance rate. In contrast, compound ZINC98606149 demonstrates a clearance rate ranging from 5 to 15, showing a comparatively higher elimination rate from the body. Therefore, these drugs must undergo rigorous laboratory testing to determine their efficacy in treating dysferlinopathy. Dysferlinopathy, a genetic disorder distinguished by the deficit or malfunction of the dysferlin protein, poses significant obstacles in terms of treatment. Consequently, evaluating possible therapies for dysferlinopathy requires careful study and evaluation within controlled laboratory environments. By subjecting these drugs to extensive testing protocols, researchers may gain valuable insights into the mechanisms of action.

High-throughput screening involves the rapid testing of large libraries of compounds against a specific target protein. This approach allows for examining thousands, or even millions, of compounds in a relatively short period.

By evaluating the compounds' ability to bind to the target protein and induce modifications in its functionality, researchers can identify lead compounds that exhibit promising activity levels. Virtual screening, on the other hand, utilizes computational methods to screen large databases of chemical compounds.

The use of sophisticated algorithms and molecular modeling techniques, researchers enhance the effectiveness, selectivity, and reduction of undesirable lead effects. It is imperative to explore the modification of their chemical structures and gain a comprehensive understanding of the intricate ligand-receptor interaction. By modifying the chemical composition of the lead compounds, researchers can enhance their therapeutic properties and minimize any adverse effects associated with their use. Furthermore, a thorough comprehension of the ligand-receptor interaction can provide valuable insights into the mechanisms underlying lead compound activity, enabling the development of more targeted and efficient therapeutic interventions. Therefore, the strategic modification of lead compounds and the elucidation of ligand-receptor interactions are promising in advancing lead compound research and improving their overall pharmacological profile [54]. By using different computational techniques, like molecular docking and molecular dynamics simulations, researchers can gain the usage of (ADD) which can be effectively facilitated through the use of comparative investigations between projected and actual pharmacological activity, along with the iterative utilization of the obtained data to enhance compound features.

The introduction of such a system is expected to transform the area, offering researchers and practitioners an invaluable tool to understand the intricacies of this complicated domain based on drug delivery systems [5] [6]. The factors that primarily impacts the distal muscle groups of the limb girdle, it is important to clinically observe particular pattern of muscle involvement. The etiology of these disorders can be attributed to the reduction of the dysferlin [7] protein from the plasma membrane of muscle fibers, leading to disruptions in the movement of vesicles and membrane repair. Consequently, both conditions are occasionally referred to as "dysferlinopathy " within the general population, the incidence of progressive muscular dystrophy is thought to be four occurrences per 100,000 individuals [7]. This condition has three primary subtypes, specifically DMD, accounting for 60% of cases, LGMD, accounting for 30% of cases.Identically, facioscapulohumeral dystrophy [55], accounting for 10% of cases.

The frequency of LGMD2B in the Japanese community is far greater, making up around 18% of all LGMD cases, comparable to other nations [8] [56]. The absence of effective therapy for dysferlinopathy

necessitates the development of an improved approach to involve the treatment of such kind of genetic condition [57].

In concern of economic efficiency of CADD is also improved through the collaboration of comparative experiments and iterative utilization of data. Researchers can lower the number of costly and time-consuming testing trials by validating computational models and developing compound features. This saves resources and speeds up the drug discovery and development process. Next, by combining comparative experiments between projected and actual pharmaceutical in computer-aided drug design, two fundamental methodologies have emerged as prominent approaches: structure-based drug design (SBDD) and ligand-based drug design (LBDD). SBDD depends on the information and analysis of protein structures to guide the development of novel compounds. At the same time, LBDD leverages information about ligands and their activities to design compounds interacting with protein structures. By examining the three-dimensional structure of target proteins, researchers can identify potential binding sites and gain insights into the molecular interactions between ligands and proteins. This knowledge is then employed to guide the rational design of compounds that can effectively interact with the specific protein, thereby modulating activity and potentially leading to therapeutic benefits. In contrast, ligand-based drug design emphasizes analyzing known ligands and their actions to inform the design of new compounds. By studying the structure-activity relationships (SAR) of ligands that have demonstrated desirable pharmacological properties, researchers can identify key structural features and properties that contribute to their biological activity. This information is then utilized to design new compounds with similar structural motifs or properties to achieve comparable or improved pharmacological effects. Both SBDD and LBDD methodologies have their strengths [58] [59].

In the context of dysferlinopathy, it is interesting that ZINC98606149 illustrates the highest value for plasma protein binding (PPB), thereby classifying it as a drug with a low therapeutic index for this particular condition. The BBB (blood-brain barrier) penetration is also observed in these drugs, making them suitable for treating dysferlinopathy. The analysis of the drugs' metabolism rate has revealed that they can be categorized as drugs ideal for therapeutic applications as inhibitors of dysferlin in dysferlinopathy. This finding indicates that these drugs possess the potential to modulate dysferlin levels thereby and adequately contribute to the management and cure of dysferlinopathy.

Further investigation and clinical trials are necessary to validate and expand upon these initial observations, ultimately paving the way for developing novel therapeutic interventions targeting dysferlin-related disorders.

Declarations
Author contributions
This manuscript supports Ms. Shumaila Azam for her doctoral dissertation completion, in which Dr. Sahar Fazal has supervised the whole research work.

Conceptualization, Methodology, Writing and Software: Shumaila Azam and Gulnaz Parveen
Validation and Review: Dr. Sahar Fazal

Ethics approval and consent to participate
Not applicable.

Availability of data and materials
The data used in this manuscript is taken from open-source databases e.g. Protein Databank, PubChem, and ZINC Database. All these sources are freely accessible to the researchers.

Conflict of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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