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Aging Genome Modification and Editing using the Crispr-Cas9 system: Anti-Alzheimer Study by Docking Methods

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Abstract: Cas-9 is an enzyme that uses CRISPR sequences as a guide for detecting and separating a part of the genome which are complementary to the CRISPR sequence. Cas9 (CRISPR-associated protein 9, formerly called Cas5, Csn1, or Csx12) plays an important role in the human immune system against DNA viruses and is also utilized in genetic engineering approaches. They are able to cut a part of the DNA sequence in genome editing. CRISPR-Cas-9 editing was established by Emmanuelle Charpentier and Jennifer Doudna (Nobel Prize in Chemistry in 2020). CRISPR has been edited for making transcription items that permit researchers to activate specific genes. There are two categories of CRISPR-Cas; category 1 consists of multiple Cas proteins for degrading foreign nucleic bases. Category 2 consists of a single huge Cas protein for the same role. Aging results from a lifetime of stochastic destruction of tissues and cellular ingredients. Increasing age parallel causes a decrease of immunity and any inflammation related to reflecting incidents of cellular and tissue damage as a function of a lifetime. The DNA sensing signaling is activated via wrong placed cytosolic, which initiates the innate immune responses. Micronuclei are completely related to aging and affect aging due to always occurring in several aging syndromes and cancer. Therefore, micronuclei may present a mechanistic link among genome instabilities, innate immune activation, and a few hallmarks of aging tissues with the different drug properties of Verubecestat, Donepezil, Memantine, galantamine, Tacrine, Exelon, Rivastigmine, 7-MEOTA, and Acyclovir. Among them, Tacrine was found to have the highest (negative) binding energy and was further subjected to molecular dynamics (MD) simulation analysis.

Keywords: Alzheimer; Tacrine; Crispr-Cas9 system; Nobel Prize in Chemistry in 2020; docking methods

1. Introduction

1.1. CRISPR.

CRISPR is a section of DNA in the genomes of prokaryotic organisms [1-5], which are extracted from DNA segments of bacteriophages that had firstly infected the prokaryote. Since they are applied for detecting and removing a part of the genome during any infections, these sequences also play an important role in the antiviral defense system of prokaryotes. CRISPR has appeared in more than 45% of bacterial genomes and approximately 85% of archaea genomes; in addition, Cas-9 is an enzyme that uses CRISPR sequences as a guide for detecting and separating a part of the genome, which are complementary to the CRISPR sequence [6, 7].

Both Cas-9 enzymes and CRISPR make the foundation of CRISPR-Cas-9 technologies for any modification and editing genome of various organisms. These technologies have various applications, including bioresearch, biotechnology, and treatment [8-10]. CRISPR-Cas-9 editing was established by Emmanuelle Charpentier and Jennifer Doudna (Nobel Prize in Chemistry in 2020). The first CRISPR phenomenon was discovered accidentally by Yoshizumi at Osaka University (1987) with cloned part of *Escherichia cologne* during ribozyme conversion of alkaline phosphatase from the genome [11]. The relation of this phenomenon due to the repeats without interspersed different sequences was unusual, and there was no answer to the function of the interrupted clustered repeats. In 1993, several articles were published in Holland about a cluster of interrupted direct repeats (DR) in that bacterium. They discovered the diversities of the direct repeats among different tuberculosis strains and used this ability to design a kind of genetic typing known as the spoligotyping method today [12-14]. This is because the clustered repeats played a play in properly separating replicated genome into daughter DNA during cell division. Both plasmid and chromosome with equal repeated arrays cannot be placed in *Haloferaxe volcanii*, the first concept of CRISPR. Mojica and his colleagues established an algorithm for searching this kind of genome, and they predicted interrupted repeats in twenty species of bacteria belonging to the same group [15]. Due to inter distancing of those genomes, Mojica supposed these genomes might occur through a short, regularly spaced repeated, abbreviated as SRSR. Tang exhibited that the CRISPR sequences from DNA of the Archaeoglobus can be transcribed into large RNA that was processed into small RNAs [16]. Research in yogurt exhibited that Streptococcus, after iterative phage reactions, can increase the phage resistance because of CRISPR sequences [17]. A basic concept of CRISPR explained by Jansen's research in the prokaryote repeat cluster was done through an ensemble of homologous sequences of a CRISPR-associated where called CAS gene. Firstly, four have been recognized (CAS 1-4) [18]. The 3 basics of CRISPR are exhibited in Figure 1, cas genes, a leader sequence, and a repeat-spacer array.

1.1. The properties of Cas-9 and its function.

The endonuclease of the Cas-9 is included in a four-segment consisting of crRNA and trans-activating CRISPR RNA [19, 20]. Doudna and Charpentier re-structure this endonuclease into a more manageable pair of components by fusing two RNA into one structure to find and cut the DNA target specified by the guide RNA(Nobel Prize in Chemistry in 2020). Through manipulating the guide RNA, the artificial Cas-9 could be programmed to target any DNA sequence for cleavage [21]. Zhang and Church exhibited the concept of genetic modification in cell cultures using CRISPR-Cas9 [22, 23]. CRISPR has been edited for making transcription items that permit researchers to activate specific genes [24]. The CRISPR-Cas9 structure has exhibited an effective gene modification in humans and made a successful mutant in 28 out of 54 embryos, and 4 out of them were completely reorganized using a donor template given by the researchers. The researchers exhibited that during DNA recombination, the endogenous sequence is replaced with a donor exogenous for any DNA repairing in humans and particular stem cells [25].

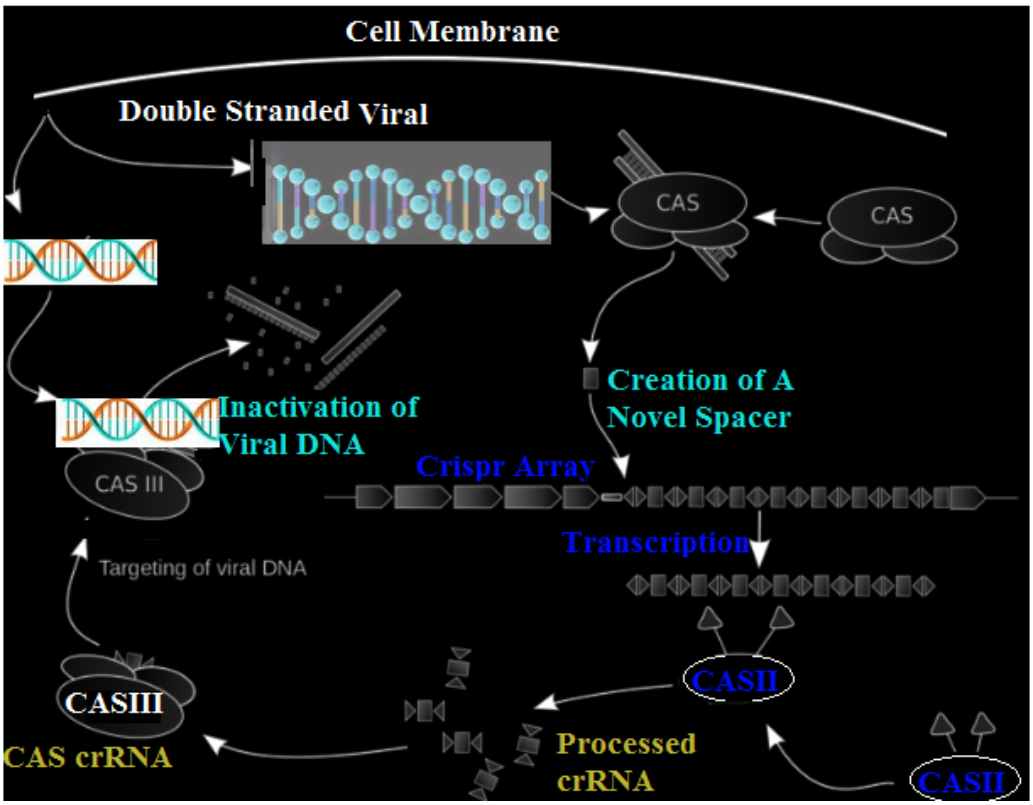

Figure 1. Diagram of the CRISPR prokaryotic antiviral defense mechanism.

The Cas12a was discovered in 2015 for CRISPR/Cpf1 from the bacterium Francisella [26], which includes several differences to Cas9 structure and is based on cutting DNA with relying on providing alternative targeting sites Cas9 and CRISPR RNA for successful targeting. It is notable that Cas9 needs both crRNA and a trans-activation crRNA for the stability of its structure [27]. This change could be giving several advantages to the Cas12a system compared with Cas9. For instance, small crRNAs are ideal for multiplexed genetic modified such as DNA assembly, which is a target-specific than traditional Restriction Enzyme cloning [28]. Therefore, Cas12a can be cleaved DNA base pairs in the section of the PAM site due to no disruption, so Cas12a causes multiple cycles of DNA cleavage. Therefore, Cas12a can be cleaved DNA base pairs in the section of the PAM site due to no disruption, so Cas12a causes multiple cycles of DNA cleavage. Repeated cycles might make a large genomic modifying [29, 30]. An amazing specific of Cas12a, compared with Cas9, is its target that for Cas12a, it might be collateral cleavage that is known as trans-cleavage [30-31]. Six years ago, the nuclease Cas13a or C2c2 was explored from the Leptotrichia, an RNA-guided endonuclease that does not cleave DNA. Cas13a is guided by its crRNA to an ssRNA target that cleaves the goal center ofter binding [32]. CRISPR sequences consist of an AT-rich and are separated by identical spacers [27].

Their ranges are from 29 to 38 base pairs [33]. The sizes of spacers in various CRISPR sequences are generally 30 to 40 base pairs [34]. These spacers could appear quickly as a section of the immune reflation for any phage infection [35]. It is notable that more than 55 units of the repeat-spacer sequence in each CRISPR array [34, 35]. Several CRISPR RNA is shown in Figure 2. The CRISPR/Cas9 is a way for double-stranded breaking in DNA that can knock out any gene through non-homologous repairing routes and repair it through homology. The CRISPR/Cas9 complexes need programming of RNA against goals. Gene drives that are a suitable method could increase the particular genome for prevailing it from its population.

Generally, genomes occasionally gain a 50% chance of being inherited. This mechanism is evaluated as natural 'gene drives' that first is discovered in a single organism. Artificial gene drives can be a very useful way to control several diseases. Apart from disease control, CRISPR/Cas9 can be applied to research as an important platform for molecular genetic studies.

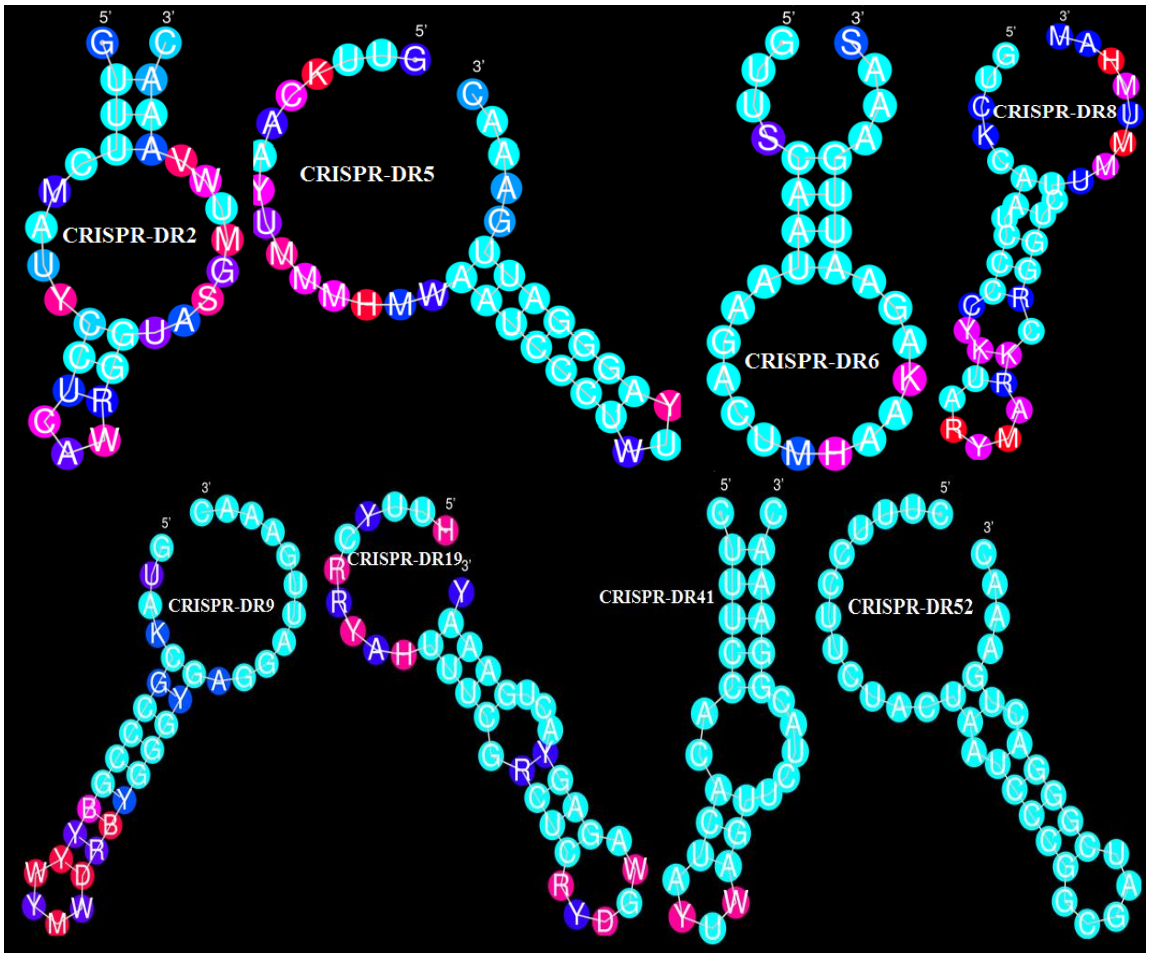

Figure 2. CRISPR-DRn- RNA (n=2,5,6,8,9,19,41 and 52).

1.2. Cas-n genes and CRISPR subtypes.

The gene clusters of Cas sequence are occasionally placed next to CRISPR repeated arrays. The 95 Cas genes are merged into 36 groups based on the same as the encoded proteins. 10 of the 36 groups make the Cas root that consists of the protein groups Cas1 through Cas9. Each CRISPR-Cas locus has at least one gene inside the Cas sequence root [36]. There are two categories of CRISPR-Cas; category 1 consists of multiple Cas proteins for degrading foreign nucleic bases. Category 2 consists of a single huge Cas protein for the same role. The categorie1 is divided into groups of I, III, and (IV), while the categorie2 is divided into groups of II, V, and VI [37].

The six above mentioned are divided into 19 subgroups, and each of them is characterized by a signature gene [38]. Although numerous CRISPR-Cas items have a Cas1 protein, exceptions exist due to module shuffling [39]. Most organisms of multiple CRISPR-CAS could share components, and their CRISPR/Cas sub-groups are a subject of gene transfer during microbial evolution [40, 41]. Signature genes and their specific functions for the major and minor CRISPR-cas categories are listed in table1.

Table 1. Signature genes and their putative functions for the major and minor CRISPR-cas types.

Category	Cas group	Cas subgroup	Signature protein	Function	References
1	I	1-A	Cas8a	Cas8 is a Subunit of the interference module that is important in targeting invading DNA by recognizing the PAM sequence.	[39, 44]
		1-A	Cas5	Cas5 is required for the processing and stability of crRNAs	[42, 43]
		1-B	Cas8b	Cas8 is a Subunit of the interference module that is important in targeting invading DNA by recognizing the PAM sequence.	[42 ,43]
		1-C	Cas8c		
		1-D	Cas10d	contains a domain homologous to the palm domain of nucleic acid polymerases and nucleotide cyclases	[45, 46]
		1-E	Case1		[45, 46]
		1-E	Case2		[45, 46]
		1-F	Csy1 Csy2 Csy3	Not determined	[39]
		-	Cas3	Single-stranded DNA nuclease (HD domain) and ATP-dependent helicase	[42, 43]
	III	-	Cas10	The homolog of Cas10d and Cse1. Binds CRISPR target RNA and promotes stability of the interference complex	[46, 48]
		IIIA	Csm2	Not Determined	[39]
		IIIB	Cmr5		[39, 40]
		IIIC	Cas10 or Cs×11		[39, 40]
		IIID	Cs×10		[39, 40]
		IIIE			[47]
		IIIF			[47]
	IV	-	Csf1		[47]
		IV-A			[47]
		IV-B			[47]
		IV-C			[47]
2	II	-	Cas9	Nucleases RuvC and HNH together produce DSBs, and separately can produce single-strand breaks. Ensures the acquisition of functional spacers during adaptation.	[49, 50]
		II-A	Csn2	Ring-shaped DNA-binding protein. Involved in primed adaptation in Type II CRISPR system.	[51]
		II-B	Cas4	Endonuclease that works with cas1 and cas2 to generate spacer sequences	[52]
		II-C		Characterized by the absence of either Csn2 or Cas4	[53]
	V	-	Cas12		[54]
		V-A	Cas12a (Cpf1)		[47]
		V-B	Cas12b (C2c1)		[47]
		V-C	Cas12c (C2c3)		[47]
		V-D	Cas12d (CasY)		[47]
		V-E	Cas12e (CasX)		[47]
		V-F	Cas12f (Cas14, C2c10)		[47]
		V-G	Cas12g		[47]
		V-H	Cas12h		[47]
		V-I	Cas12i		[47]
		V-K	Cas12k (C2c5)		[47]
		V-U	C2c4, C2c8, C2c9		[47]
	VI	-	Cas13	RNA-guided RNase	[53, 54]

By attacking a bacteriophage to a microbe, the first step of the immune response is to capture phage DNA by the CRISPR locus in the position of the spacer. By mutation studies, it was confirmed that both Cas1 and Cas2 are contained in the spacer. The hypothesis shows that removal of cas1 or cas2 can be locked spacer acquisition without affecting CRISPR immune reaction. Although Cas1 has various polypeptides and proteins, their crystal structures are metal-dependent nucleases/integrate that bind to DNA strands independently [40]. These proteins of cas2 have been structured as either single strands of RNA or double strands of DNA [41-55].

1.3.Cas9 structure and properties.

Cas9 (CRISPR-associated protein 9, formerly called Cas5, Csn1, or Csx12) plays an important role in the human immune system against DNA viruses and is also utilized in genetic engineering approaches. They are able to cut a part of DNA sequence in genome editing (Nobel Prize in Chemistry in 2020). Structurally, it is a dual RNA-guided endonuclease with a Clustered, Regularly Interspaced system in *Streptococcus pyogenic* [56, 57]. *S. pyogenes* keep the codes for Cas9 to later cleave foreign DNA, such as invading bacteriophage DNA or plasmid DNA [58]. Consequently, it makes this interrogation via unwinding foreign DNA and evaluating for sites around 20 base pair regions of the guide RNA. If the DNA is completed, it can be separated from the invading DNA. These cleavages might help gene inactivation through non-homologous end-joining and homologous recombination, respectively, in many laboratory model organisms. Behind nucleases and Transcription activator-activated nuclease proteins, Cas9 seems to be an important tool in genome editing. It has been considered in genetic studies in recent years due to its ability to cleave in any guide RNA sequence [58]. The character of Cas9 stems from the RNA/DNA converting and not editing to the protein itself, and the genetic engineering of Cas9 to DNA is straightforward [59]. Items of Cas9 attached to DNA without any cleave cognate can transcriptional to the related DNA sequences [60]. Native Cas9 needs a guide RNA combination of two disparate RNAs that associate. Cas9 targeting has been facilitated via the engineering of single guide RNA. Researchers have suggested that Cas9-based gene drives can be capable of modifying the genomes of organisms [61]. In 2015, Cas9 was used to modify the genome of human embryos for the first time [62]. The dCas9, or endonuclease deficient Cas9, is applied for editing genome translation during the expression of the desired section of a DNA sequence. Its function is based on a special activity mode. Gene translation is inhibited during nucleotides adding to the RNA chain at the same time as terminating elongation. Consequently, this process has been seen at any sequence-specific guide RNA molecule. As dCas9 can regulate gene expression, its action is amplified even more when it is used in conjunction with repressive chromatin modifier domains [63].

1.4. Aging genome modification.

1.4.1. Cytosolic, A potential source of anti-aging.

Aging is a result of a lifetime of stochastic destruction of tissues and cellular ingredients. Increasing age parallel causes a decrease of immunity and any inflammation related to reflecting incidents of cellular and tissue-damaging as a function of a lifetime. The DNA sensing signaling is activated via wrong placed cytosolic, which initiates the innate immune responses. DNA damage destroys nucleus integrity and mitochondrial, resulting in inflammatory responses to the aging process [64-66]. Studies in human biology have shown

that a state of systemic and chronic inflammation appears in any part of body tissues parallel with increasing age [66]. Aging is the most risk item for chronic diseases like diabetes, cardiovascular problems, cancer, and kidney disease. Aging frailty usually depends on a clinical position and vulnerability in older adults to risk of disability and mortality, which generally appear with high inflammatory markers containing damaging protein [67]. The aging biological processes include several tissue mechanisms further influenced by inherited genetic predisposition and lifestyle. Aging-related altered chronic inflammatory reactions can be interpreted as a result of disturbances in cellular and tissue homeostasis due to persistent cellular and tissue damage [68]. Inflammation can also be started via situations that are usually misplaced cellular components, such as misplacing of cytosolic nuclear and mitochondrial DNA. Therefore perturbation agent in the cells appears to potent inflammation [69]. A correct cellular reaction to cytosolic DNA prevents a mutation in enzymes that stop the accumulation of cytosolic DNA or prevent the signaling systems that respond to cytosolic DNA [70]. Many kinds of cytosolic sensors have been recognized, such as melanoma 2, cyclic GMP-AMP synthase (cGAS), and interferon-γ-inducible 16, and these sensors activate some the inflammatory [71]. IFI16 cytosolic from various tissue is able to activate and trigger pyroptosis in humans. It can interact with cGAS for innate immune response to exogenous viruses [72]. The cytosolic sensor and its effector of Interferon Genes initiate a major signaling route in the defense against any infection cGAS-STING signaling. Its central function in inflammation can react to misplaced cytosolic in both immune and non-immune cells. Activated cGAS send the second signal, which binds to the trans-Golgi circuit and transcription factor IRF3. This binding promotes the expression of type I interferon (IFNI) and inflammatory cytokines, and chemokine [73] (Figure 3).

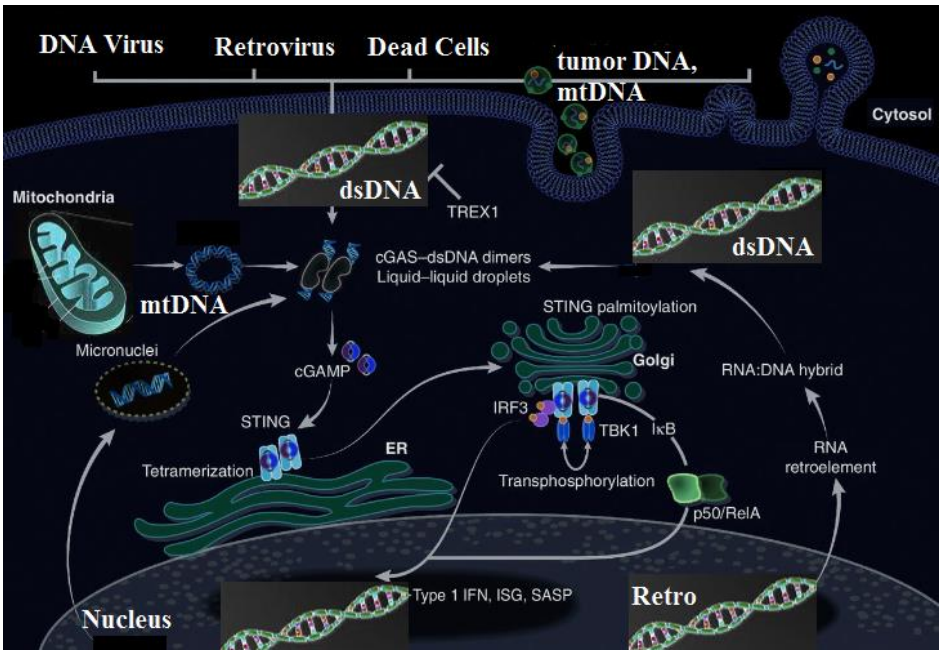

Figure 3. Activation and propagation of the cGAS-STING signaling by cytosolic self-DNA. cGAS–STING signaling in immunity. cGAS is an innate immune sensor that recognizes a diverse array of cytosolic dsDNA, including DNA with viral, apoptotic, exosomal, mitochondrial, micronuclei, and retroelement origins.

In addition, junctions and transporters allow rapid flow of cGAMP between adjacent cells. Therefore, cytosolic accumulation can activate inflammatory reactions in multiple adjacent cells, thus amplifying a local tissue inflammatory. cGAS-STING pathway activation and STING transport from the endoplasmic reticulum to the Golgi are largely controlled by

post-translational protein modifications [74-76]. Both cGAS and STING are exhibited as the stimulated genome, and their translation has been directed t via IFNI, increasing the cGAS/STING reaction to cytosolic DNA. Consequently, several forms of self-DNA are alternatively released into the cytoplasm, affecting the cGAS-STING signaling and IFN I production [77]. The keeping of the chemical properties and the genome sequence are important for life, and all living tissues have tried to repair DNA to stabilize its function and structure. Results confirm some DNAs destroyed by mutation during aging in and damaged genome can cause aging in several routes; an important one is via perturbing formal translation and persistent any problem in signaling and apoptosis during cellular aging [78]. The relation between DNA's instabilities and any inflammations is evidence that destroyed DNA can promote cytosolic nuclear activation of the cGAS-STING pathway [79]. Micronuclei are affected due to damaged mitosis and chromosomal instabilities, so PLK1-interacting helicase is needed to stabilize anaphase DNA bridges. Cells lacking PICH display chromosomal instability and increased level of micronuclei formation and consequently numerous ribonucleotides that can give rise to genome instability. Since RNAse H2 is an important enzyme for removing ribonucleotides, loss of RNase H2 causes interferonopathy, which is cGAS-STING dependent [80]. Data confirms that RNase H2 decreasing causes the increasing micronuclei formation and consequently causes the expression of interferon-stimulated genes, ISGs, which involve the cGAS-STING signaling route [81]. RNA/DNA mixed could appear transcription and replication in the nucleus and mitochondria and accumulate in RNase H2-deficient Aicardi-Gautier Syndrome patients. This may have increased the repair of highly frequent ribonucleotide DNA lesions and is essential for the maintenance of genome stability [82]. Since micronuclei are not stable (they cannot import all the necessary proteins to preserve the nuclear envelope integrity), occasional disintegration of the micronuclei membrane exposes their DNA content to cGAS, a major problem [82]. Micronuclei are completely related to aging and also affect aging due to occur always in several aging syndromes and cancer [83, 84]. Therefore, micronuclei may present a mechanistic link among genome instabilities, innate immune activation, and a few hallmarks of aging tissues [85-89].

1.4.2. Cellular senescence.

Senescence is a situation of stopping the cell cycle in somatic cells that are seemingly related to damaged cells that can develop into cancer cells [90]. It is notable that senescence is defined as an aging tissue increase in various tissues over time, which is thought to contribute to the systemic impairment in tissue function, repair, and regeneration with age. Aging phenotype is a characteristic of old cells. It relates to the expression and secretion of several pro-inflammatory cytokines, chemokines, proteases, and growth agents that decrease tissue function over time. cGAS-STING activity seems to have an important role in senescence phenotypes.

Consequently, the release of chromosome fragments into the cytoplasm due to the disruption of the nuclear envelope integrity in the old cells. In senescent cells, retro transpose elements become transcriptionally activated. In addition, the age-associated decline in mitotic fidelity gives rise to micronuclei formation, SASP expression, and cellular aging [91, 92]. Autophagy is the process of removing damaged invading pathogens in tissues. By this mechanism, cytosolic components are alternatively sent to lysosomes for destruction. There are 3 models of autophagy based on a system of types of cargo; one is macroautophagy, the second is microautophagy, and the third is chaperone-mediated autophagy [93]. It has been

explored that the autophagy phenomenon decrease with aging. In addition, in autophagy-related genes in mice, an increase in basal autophagy extends lifetime and increases health span. Genetic research has confirmed the relation of autophagy with several inflammatory and autoimmune diseases. There is a hard interaction between autophagy and the cGAS-STING route that explains autophagy in cellular defense versus pathogens. It is notable that autophagy can compromise cells' accumulated dysfunctional mitochondria with lipopolysaccharide and improve mitochondrial function by reducing age-associated inflammation. It confirms that autophagy has an important role in mitochondrial control, immunity, and inflammation, and is needed for cellular homeostasis. Such information is helpful in understanding the relative of cytosolic self-DNA in age-related inflammation in different tissues [94, 95].

2. Materials and Methods

2.1. Docking.

BIOVIA_2020 software has been used for docking, and the grids between 20-25 Å were done over the co-crystallized aging and Parkinson mutation genome. Re-docking of the molecules was done to consider docking protocols. The docked shapes were evaluated based on crystal structures of related proteins for calculating the root mean square deviation. The re-docking of macromolecules poses with 1.20Å RMSD. Lower RMSD indicates the methodologies are adequate and could be used to search for any further inhibitors. Docking was done in 3 different modes, virtual screening followed by standard-precision (SP) and extra-precision (XP) docking using the Glide program. In this research, the iGEMDOCK has been used, and the acceptable receptor can be defined for the binding site in whole protein structures. iGEMDOCK can help to quickly define the suitable binding site based on energy Gibbs amounts and docking simulation steps.

Consequently, the prediction and scores of ligands can be saved in the output routs. The optimum energy poses of each system will be outputted into the location of "best: Pose". These are based on interaction and atomic combination aspects. Interaction aspects are extracted from the macromolecule couples, and atomic structures are calculated via atomic types [96-99].

2.2. Editing Alzheimer's disease via Crispr-Cas9.

Age is known to be the greatest risk factor for developing Parkinson's. The age-related decline in midbrain dopamine-producing neurons may reach a threshold below which clinical symptoms emerge. An individual's total number of dopaminergic neurons may therefore contribute to the risk and age of onset of Parkinson's. In addition, there are numerous rare variants in single genes that are enough to produce disease. Those genetic items of Parkinson's were declared via analysis of affected families, such as SNCA, PARK7, and PRKN. Additionally, genome-wide association researchers have identified many common genetic items that individually contribute a small amount to the risk of developing Parkinson's. In the middle of this spectrum lie uncommon (but not rare) variants that exert an intermediate risk, such as GBA and LRRK2 variants (Figure 4).

Different Parkinson's subgroups should have separated in viewpoints of treatments. Common classification items for Parkinson's might be the aging situation or family history. The single genic forms of Parkinson's are rare and might not represent the same disease mechanism. For example, PRKN, PINK1, and PARK7 are all genomic regulators of mitochondrial function [100].

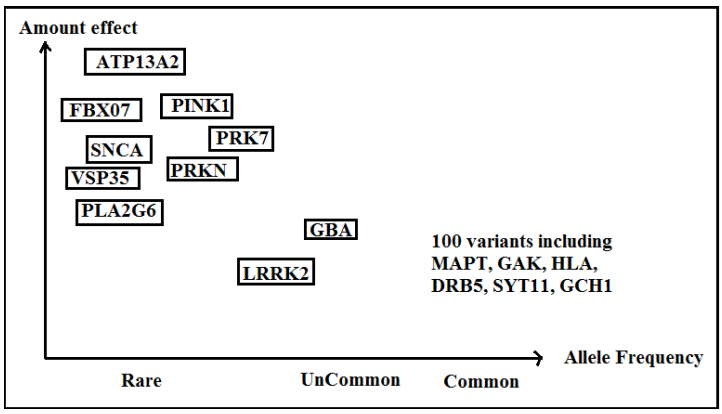

Figure 4. Genetic variants in Parkinson's disease according to the risk of Parkinson's disease.

General Parkinson's disease exerts the rare SNCA variants, supporting the existing centric model. On another side, a range of neurodegenerative diseases, same as Alzheimer's and dementia, suggest other pathways in PD pathogenesis. These factors affect the disease process and can identify causative genes and highlight biological pathways important to pathogenesis. This, in turn, can allow the development of therapeutics which target those biological pathways. This work concerns the role of CRISPR/Cas9 gene editing in managing Alzheimer's via mediated genome editing and its use against neurodegenerative disorders Parkinson's disease, Huntington's disease, and other human diseases, as is one important emerging technology for disease treatment. CRISPR/Cas9 genome editing is one of the widest and strong tools for editing incorrect genetic signatures such as Alzheimer's control due to its large potential to correct undesired gene mutations associated with any genetic disease.

2.2.1. Different strategies of CRISPR/Cas9 for genome editing.

Generally, CRISPR/Cas9 technologies have two basic components: the single-guide RNA known as sgRNA for recognizing the DNA sequence for improvement and the second is the Cas9 protein used as a scissor in DNA double strands for cutting (Figure 5). CRISPR/Cas systems are classified 1 and 2, including several Cas groups and Cas subgroups (Table 1). Category1 contains a variety of Cas proteins that work together, whereas Category 2 utilizes a single Cas protein, which makes it simple and desirable for genome editing. Among these two categories 2, the group II CRISPR/Cas9 system is the most important in pharmaceutical development. The Cas9 breaks the stop sequence after recognizing the final goal genome.

Consequently, 2 separate routes can be started to repair this break, that is, homology-directed repair (HDR) or non-homologous end joining (NHEJ) (Figure 5). NHEJ causes insertion and deletion and stops codons and/or DNA algorithms, finally resulting in an inactivated genome, while the HDR route helps replace the mutated gene with the true one [101, 102]. In CRISPR/Cas9 gene-editing method, ribonucleoprotein of Cas9 protein and sgRNA is a suitable strategy for genome editing with several advantages such as quickly starting, accurate targeting, without risk, and high editing efficiency. Moreover, the Cas9 RNP method could be used for several system organisms and cell groups, such as stem cells [103], immune cells, primary cells, etc. Recently, several items containing physical subjects and synthetic carriers can be used to use Cas9 RNP. Since, Microinjection has limited requirements, cumbersome system, and high cost, economic electroporation-based strategy to deliver Cas9 RNP system.

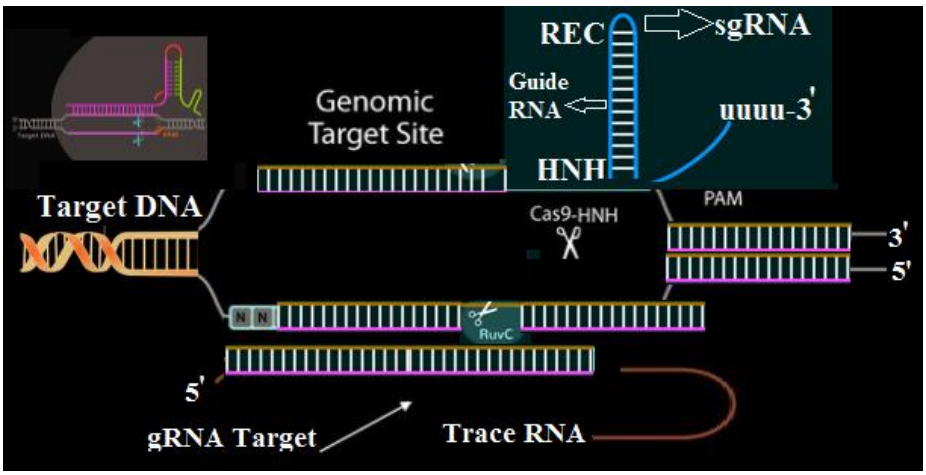

Figure 5. sgRNA (guide RNA) attaches with Cas9 in the genomic target site.

These strategies are not independent of each other. Every strategy has its advantages and limitations (Table 2). Latinize *et al*. [104] compared the several systems-based CRISPR/Cas9 into HSPCs; the analysis data exhibited that plasmid-based methods have high editing efficiency but were associated with significant cell toxicity. The RNA-mediated way has similar cell toxicity to the plasmid-based method and has less editing efficiency.

Table 2. different strategies CRISPR/Cas9 delivery format.

Delivery system	Editing efficiency	Advantages	Limitations	References
Plasmid	Moderate	Gene synthesis is simple; no need to integrate into the genome; tissue or cell-specific targeting	Low capacity	[105]
mRNA	Moderate	Transient in function; No need to integrate into the genome	Low RNA stability; delivery component individually	[106]
CRISPR/Cas9 delivered	High	Transient in function; No need to integrate into the genome	Low RNA stability; delivery component individually	[107]
Protein	High	Fast; lower off-targeting	Non-specific	[108,109]

The RNA-mediated way has similar cell toxicity to the plasmid-based method and has less editing efficiency. Although LVs-based m has minimal cell toxicity, the genome-editing efficiency is low. By contrast, RNP-based delivery of CRISPR/Cas9 had shown a suitable balance between cytotoxicity and editing efficiency.

2.2.2. Plasmid-based method.

Delivery of DNA encoding the Cas9 protein is an interesting method to exhibit the CRISPR/Cas9 system inside the cells. This machinery is a suitable way due to the relatively simple gene synthesis, and also, the synthesized gene does not need to transfer into the host cell through a plasmid. In addition, the tissue-specific delivery of the CRISPR/Cas9 is too much important for further usage. One important issue of plasmid-based delivery is that organs or cell targeting might be integrated inside the plasmid itself. In 2020, Lu *et al*. exhibited a clinical treatment via CRISPR/Cas9 PD-1-edited T cells in patients with lung tumors [110]. The plasmids expressing two sgRNAs and Cas9 edited hPD-1 plasmids were co-transfected into T cells through electroporation. After injection of these modified cells, the treatment, safety, and feasibility of therapeutic application have been evaluated by good results of CRISPR/Cas9 in cell lung tumors. In opposite to positive reports of CRISPR/Cas9 for *in vivo* DNA editing, a principal issue in the method is how to deliver high macromolecule gen to cells. Currently, Jo *et al*. found a PLGA nanoparticle pentane to deliver Cas9 into primary bone marrow-derived

macrophages. Luckily, the expression of Cas9 proteins was firstly found after one day, and TIPS fluorescence was detected in most cells [111]. Moreover, Wang *et al*. exhibited a strategy for delivering Cas9-sgPlk-1 plasmids (CPs) condensed on TAT peptide-modified same as Au/CP through electrostatic forces. This strategy for CRISPR/Cas9 mechanism is highly efficient [112].

2.2.3. RNA-based delivery.

Delivery of Cas9-encoded mRNA strategy is a usual way for the CRISPR machinery inside the cells. In contrast, gene-based delivery methods are transient in both functioning and circumventing the risks in the host genome [113]. mRNA-based is more rapid because mRNA is transcribed in minutes [106] (Figure 6).

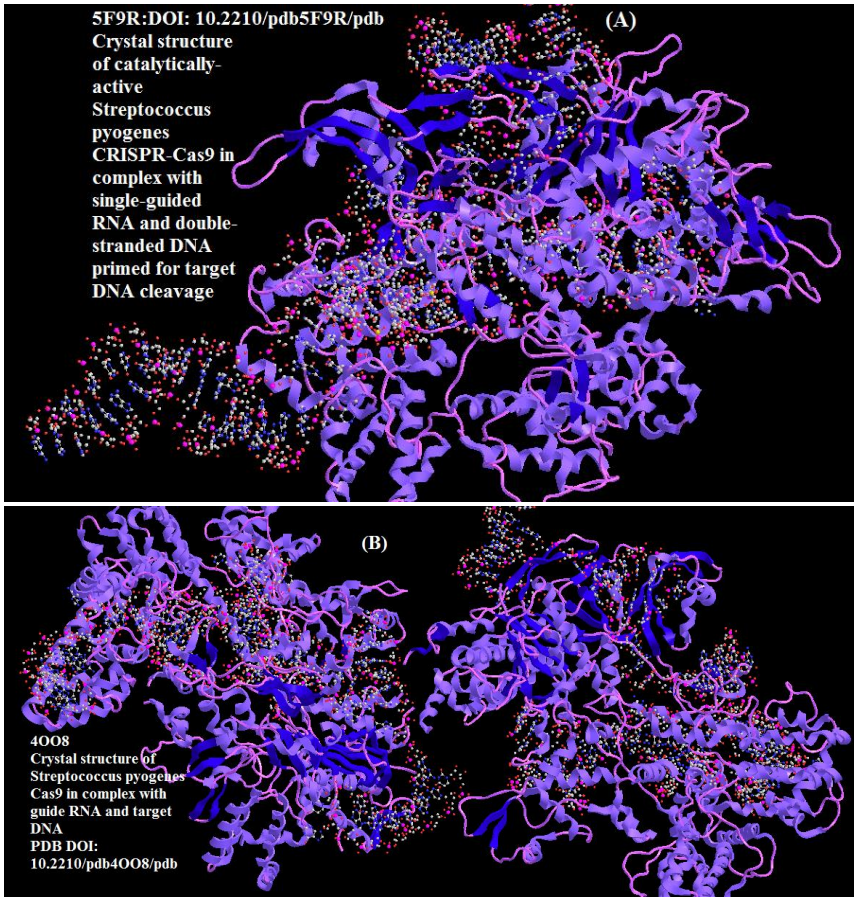

Figure 6. (**A**) Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage; (**B**) Crystal structure of Cas9 in complex with guide RNA and target DNA**.**

The challenge regarding the maintenance of RNA stability and delivery of each component of CRISPR/Cas9 individually remains. This method prospects mRNA-based CRISPR/Cas9 technology with a combinatorial delivery method. Researchers applied lipid nanomaterial to deliver Cas9 mRNA and used a virus to deliver sgRNA and a repair template. This result further shows that joint multiple delivery methods may be an effective strategy for the clinical application of CRISPR/Cas9 technology in the future, which can remedy the shortcomings of a single delivery method. To improve genome-editing efficiency by increasing sgRNA stability, further studies reported the benefits of synthetic modifications to the sgRNA [114]. This represents an effective method to overcome the stability issues associated with RNA-based CRISPR/Cas9 delivery (Figure 7).

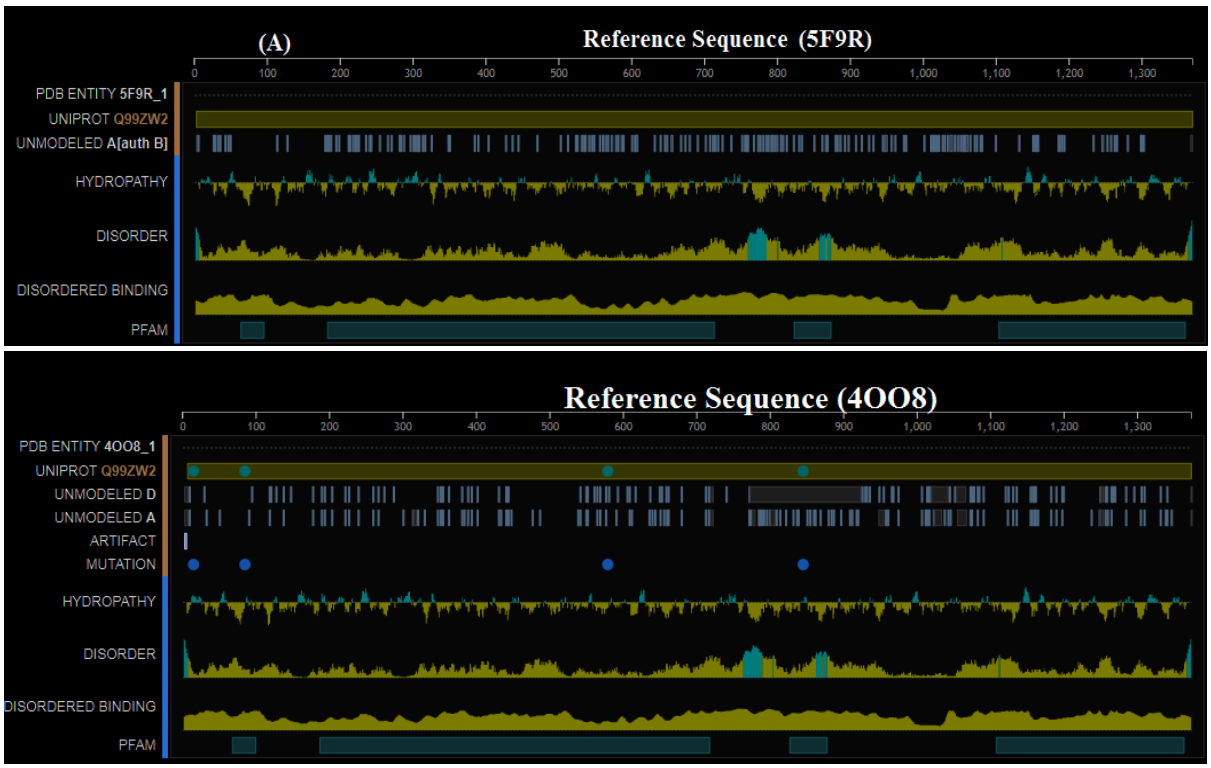

Figure 7. (**A**) Reference Sequence (5F9R); (**B**) Reference Sequence (4OO8).

2.3. Preparation of the receptor structure.

The three-dimensional (3D) Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage and Crystal structure of Cas9 in complex with guide RNA and target DNA were retrieved from the protein data bank (PDB) [115, 116] (Figure 6). A PDB file was prepared, and heteroatoms were evacuated manually. In the present study, we performed a virtual screening of a few natural compounds such as Verubecestat, Donepezil, Memantine, galantamine, Tacrine, Exelon, and Rivastigmine, 7-MEOTA, and Acyclovir by molecular docking. Cas9 was determined to interact with Tacrine through six amino acid residues, specifically Thr, Phe Glu, Met, Gln, and Ala. Tacrine complex was used for molecular docking analysis with cas9, Gasteiger charges were determined for Tacrine, and the OPLS, Amber, BIO+, and MM+ force fields were applied for energy minimization. MD simulation was performed using charmm for analyzing the energies of the cas9 -Tacrine complex. In addition, the LINCS algorithm [117, 118] was used to constrain the bond lengths. The outcomes, such as the RMSD and RMSF cas9–Tacrine complex, were analyzed according to the time-dependent behaviors of MD trajectories.

3. Results and Discussion

Based on the previous work, we have simulated this work [119-138]. The NMR position of Tacrine and Verubecestat drugs is shown in Figure 8. The docking simulation was accomplished for cell cas9-molecules interactions with several drugs via six amino acids, including Thr, Phe Glu, Met, Gln, and Ala. The binding energies and constant inhibition amount of −5.31kcal/mole and 70.61 µmol for Tacrine and −4.55kcal/mole and 65.32 µmol for Verubecestat have been calculated, respectively. The mathematical mean square deviation (RMSD) curves were estimated via molecular dynamic simulation for the Cα backbone of the cas9–drugs molecules complexes. It exhibited that some of the complexes were more stable

than others. The complexes make an equilibrium situation during the initial simulation phase and then remain stable over 40 ns. The RMSD amounts of the complexes slowly increased for 10 ns and then stabilized between 15–60 ns, as displayed in Figure 9. Root mean square fluctuation (RMSF) data for all residues in the cas9-Tacrine complex was estimated in Figure 9. The radiuses of gyration (Rg) amount of the complex backbone were determined for a 55 ns trajectory, and it was found that the cas9-Tacrine complex was stable and densely packed.

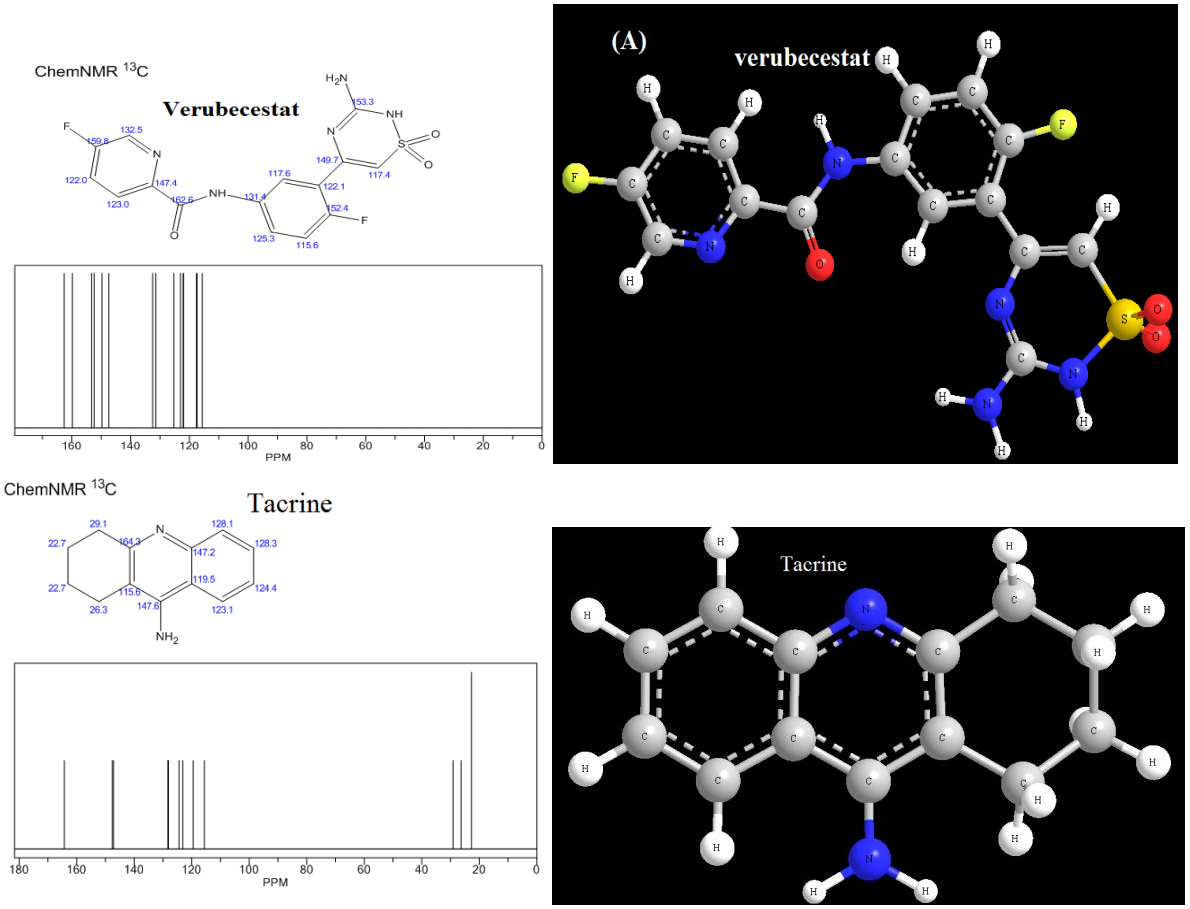

Figure 8. NMR Shielding of Tacrine and Verubecestat.

The different drug properties of Verubecestat, Donepezil, Memantine, galantamine, Tacrine, Exelon, Rivastigmine, 7-MEOTA, and Acyclovir properties, are calculated. In this work, six amino acid residues, Thr, and Phe Glu, Met, Gln, and Ala of cas9-Tacrine, simulation are applied to obtain binding mode information between receptors and ligands. Here, we applied Tacrine and Rivastigmine as control molecules to validate receptor-ligand interactions. Some other molecules appeared to have positive effects on the improvement of cognitive function and curing of Alzheimer's disease [139-143], and some of the themes exhibited significant protective effects on neuronal cells in Alzheimer's disease [144-153]. The free energies of human cas9-molecules binding have been calculated. In the present work, Thr, Phe, Glu, Met, Gln, and Ala of cas9-Tacrine residues, of cas-9, ligand: H35-A: Ala:O and A:Thr: N-ligand. The donor, H35 of Tacrine, interacted with the O ofAla:O, whereas the nitrogen of Thr: N-ligand interacted with acceptor N5 of Tacrine. The total interacting surface area of the cas9–Tacrine complex was 425.16 Å2.

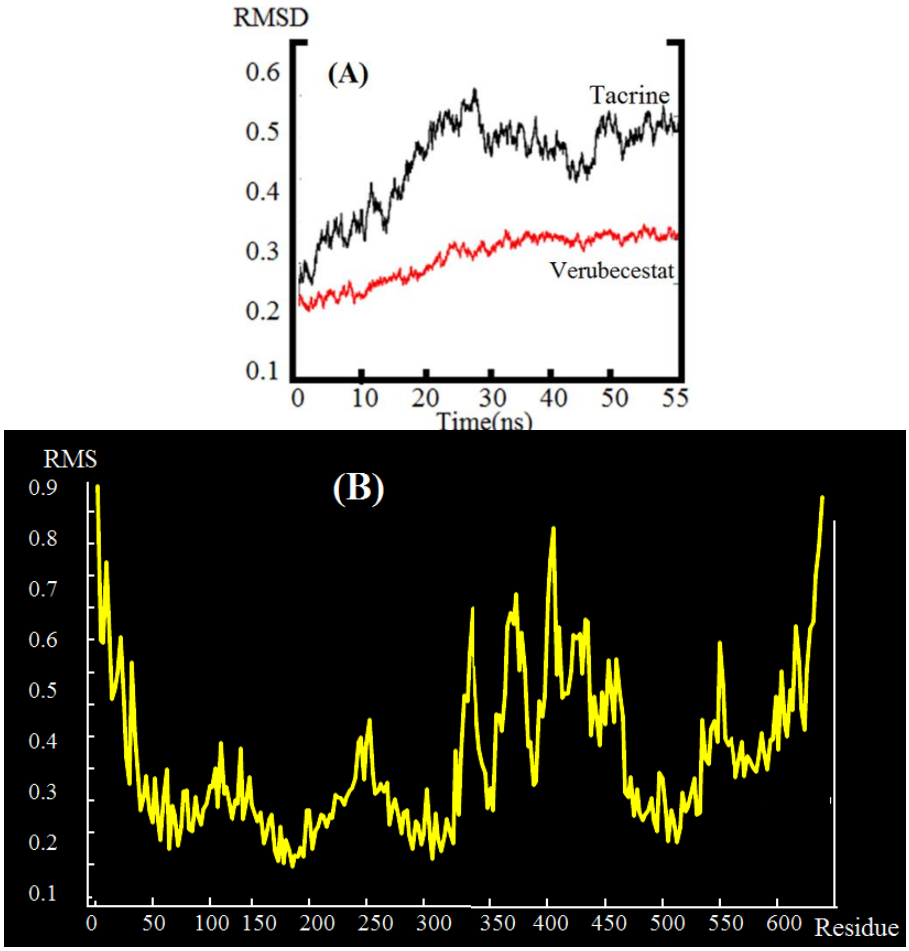

Figure 9. (**A**) The root mean square deviation of cas9- Tacrine and Verubecestat; (**B**) The root mean square fluctuation of cas9- Tacrine.

4. Conclusions

In this study, the binding among the compound Verubecestat, Donepezil, Memantine, galantamine, Tacrine, Exelon, Rivastigmine, 7-MEOTA, and Acyclovir and human cas- 9 were studied using a combination of molecular docking and molecular dynamics methods. It is hoped our findings will be found useful by researchers for discovering the reason and main root of Alzheimer's disease.

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Conflicts of Interest

The authors declare no conflict of interest.

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