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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INVESTIGATION OF THE INHIBITORY POTENTIAL OF SOME ANTIVIRAL AGENTS ON HUMAN TELOMERASE BY MOLECULAR DOCKING AND MOLECULAR DYNAMICS SIMULATION STUDIES

BAZI ANTİVİRAL AJANLARIN İNSAN TELOMERAZ ENZİMİ ÜZERİNDEKİ İNHİBİTÖR POTANSİYELİNİN MOLEKÜLER KENETLENME VE MOLEKÜLER DİNAMİK SİMÜLASYON ÇALIŞMALARI İLE ARAŞTIRILMASI

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ABSTRACT

Objective: *This study investigated the anticancer effects of nucleoside and non-nucleoside reverse transcriptase inhibitors drugs by computational methods. The study aimed to evaluate the binding capacity of these drugs on the telomerase essential N-terminal (TEN) domain of telomerase reverse transcriptase (TERT). Molecular docking was used to assess the drugs' binding potential to the TEN domain. The stability of the protein-drug combination obtained from the docking method was assessed using molecular dynamics (MD) simulation.*
Material and Method: *The TEN domain of TERT's crystal structure was obtained from the Protein Data Bank (PDB). The crystal structure identified by the PDB code 2B2A has a resolution of 2.2 Å. The molecular docking was performed using AutoDock Vina. The complexes were visualized using Biovia Discovery Studio. The MD simulation was conducted using GROMACS 2020 as indicated. An MD simulation was conducted for 200 ns on both the complexes and the free protein. The RMSD (root mean square deviation) of the backbone protein and the molecules in relation to the backbone protein, RMSF (root mean square fluctuation), and Rg (radius of gyration) were shown via Qt Grace.*
Result and Discussion: *Doravirine, Etravirine, Rilpivirine showed higher binding affinity to the TEN domain compared to the reference TERT inhibitor, BIBR1532, based on the docking investigation. The MD simulation analysis showed that the protein-Doravirine complex had the highest stability in remaining within the protein's binding pocket. On the contrary, the protein-Rilpivirine complex decreased stability, potentially causing the ligand to not to stay within the binding site. Doravirine was found to inhibit the TEN domain in the computational study. Therefore, the design and synthesis of novel doravirin derivatives is being considered because of the potential anticancer activity of doravirin in inhibiting the TEN domain of TERT.*
Keywords: *MD simulation, molecular docking, NNRTI, NRTI, TERT*

ÖZ

Amaç: *Bu çalışmada, nükleozid ve non-nükleozid ters transkriptaz inhibitörü ilaçların, antikanser etki potansiyeli hesaplamalı yaklaşımlar kullanılarak araştırılmıştır. Bu amaçla, bu ilaçların*

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telomeraz ters transkirptaz (TERT)'ın telomeraz temel N-terminal (TEN) alanına bağlanma potansiyeli araştırılmıştır. İlaçların TEN alanına bağlanma potansiyeli için moleküler yerleştirme çalışması yapılmıştır. Moleküler yerleştirme sonucu elde edilen protein-ilaç kompleksinin kararlılığı moleküler dinamik (MD) simülasyonu ile değerlendirilmiştir.

Gereç ve Yöntem: *TERT'in TEN alanı kristal yapısı için Protein Veri Bankası (PDB) kullanılmıştır. 2,2 Å çözünürlüğe sahip PDB kodu 2B2A kristal yapı kullanımıştır. Moleküler yerleştirme çalışması için AutoDock Vina programı kullanılmıştır. Kompleksler Biovia Discovery Studio kullanılarak görselleştirilmiştir. MD simülasyonu GROMACS 2020 kullanılarak gerçekleştirilmiştir. Hem kompleksler hem de serbest protein üzerinde 200 ns boyunca bir MD simülasyonu gerçekleştirilmiştir. Omurga protein ve moleküllerin, omurga yapısına göre RMSD (kök ortalama kare sapması), RMSF (kök ortalama kare dalgalanması) ve Rg (dönme yarıçapı), Qt Grace ile gösterilmiştir.*

Sonuç ve Tartışma: *Moleküler yerleştirme çalışması sonucunda, Doravirin (bileşik 3), Etravirin (bileşik 6) ve Rilpivirin'in (bileşik 9) referans TERT inhibitörü BIBR1532'ye kıyasla TEN alanına daha yüksek bağlanma potansiyeli ile bağlandığını ortaya koymuştur. MD simülasyon çalışması ile, protein-Doravirin kompleksinin proteinin bağlanma cebindeki en yüksek stabiliteye sahip olduğu gösterilmiştir. Öte yandan, protein-Rilpivirin kompleksinin kararlı olmaması nedeniyle bağlanma cebinde kalmama ihtimali bulunmaktadır. Yapılan çalışma, Doravirin'in TEN'i inhibe edebileceğini göstermiştir. Bu nedenle, Doravirin'in TERT'in TEN alanını inhibe ederek antikanser potansiyel gösterebilme ihtimali nedeniyle Doravirin türevi yeni bileşiklerin tasarlanması ve sentezlenmesi düşünülmektedir.*

Anahtar Kelimeler: *MD simülasyon, moleküler yerleştirme, NNRTI, NRTI, TERT*

INTRODUCTION

People with HIV infection may have earlier development of age-related diseases due to accelerated aging processes caused by the virus. The list of HIV-associated non-AIDS disorders is expanding. Many non-AIDS disorders are linked to both increasing age and chronic inflammation. These disorders comprise cardiovascular disease, several malignancies, osteoporosis, liver disease, renal disease, and neurocognitive decline [1]. It is uncertain if the increased risk of these consequences is due to a precipitated aging process, issues emerging at earlier stages of life, or a caused emphasized aging process [2].

Antiretroviral drugs may lead to telomere shortening, which could contribute to accelerated aging in HIV-infected patients [3]. Shortened telomere length in peripheral blood mononuclear cells (PBMCs) is closely associated with age-related disorders such as cardiovascular diseases and dementia [4,5].

Telomerase is an enzyme that exists in organisms that exist that generates new DNA repeats at the termini of linear chromosomes [6]. The telomerase reverse transcriptase (TERT) and telomerase RNA (TER) both constitute the catalytic center of the telomerase enzyme [7,8]. A special region of its intrinsic RNA known as TER acts as a template for nucleotide incorporation by TERT. A comprehensive array of biochemical and cell biology studies have been conducted to evaluate the inhibitory effects of reverse transcriptase (RT) inhibitors for HIV, with particular focus on the structural similarity between the RT domains of HIV RT and TERT [9]. By adding TTAGGG sequences repeatedly to chromosomal ends, telomerase inhibits the progressive degradation of telomeres that occurs during cell division. Due to their structural and molecular similarity with HIV reverse transcriptase [10], NRTIs may inhibit telomerase.

Previous studies have demonstrated that Zidovudine inhibits the activity of telomerase and induces telomere shortening in human breast cancer cells [11], colon cancer cells [12], and leukemia cells [13]. The evaluation of Zidovudine-induced telomerase inhibition was conducted on a human hepatoma cell line [14], while cervical cancer cells exhibited telomere shortening [15].

Previous research has demonstrated that NRTIs can inhibit human telomerase utilizing the same approaches as they use to inhibit HIV RT. Repeated exposure of telomerase-positive human cells to NRTIs can lead to inadequacies in maintaining telomere length because of the inhibition of telomerase by these drugs.

This work involved constructing molecular docking and molecular dynamics simulation studies to investigate the inhibitory effects of NNRTIs/NRTIs on the human telomerase enzyme.

MATERIAL AND METHOD

Molecular Docking

The crystal structure of the TEN domain of TERT was retrieved from the protein data bank (PDB). The crystal structure with a PDB code of 2B2A has a resolution of 2.2 Å [16]. The possible binding pocket of the structure was predicted through CASTp first [17]. Then, the grid box for the molecular docking was specified based on the estimated pocket. The molecules were downloaded from the PubChem database [18]. The molecular docking was undertaken by using AutoDock Vina as used earlier. The resulting complexes were visualized through Biovia Discovery Studio [19,20].

Molecular Dynamics Simulation

The stabilities of the TEN domain-drug complexes, which were procured from the docking, were explored through MD simulation. Then, the stability of the complexes was compared to the stability of the unbound protein. The MD simulation was undertaken by using GROMACS 2020 as described in earlier studies. For the complexes and the unbound protein, MD simulation was run for 200 ns. Thereafter, RMSD (root mean square deviation) of the backbone protein and the molecules in relative to the backbone protein, RMSF (root mean square fluctuation), and Rg (radius of gyration) were drawn via qt grace. Then, the resulting plots were analyzed accordingly [21,22].

RESULT AND DISCUSSION

Molecular Docking

The binding potential of the selected antiviral drugs to the TEN domain of TERT was explored through molecular docking. Before proceeding to the docking, the binding pocket was estimated via CASTp (Figure 1). Thereafter, the binding of a TERT inhibitor, BIBR1532, to the crystal structure (2B2A) was investigated. This was performed to validate the docking protocol and set a benchmark for the interaction of the antiviral drugs under investigation. BIBR1532 is a selective potent TERT inhibitor with an IC_{50} value of 0.093 μM [23,24]. The docking study showed that the ligand interacted with the TEN domain via three conventional hydrogen bonds (Arg16(2), Ser28), pi-pi (Ser28), pi-sigma (Ala29), pi-ion (Asn191), and pi-alkyl (Lys31) interactions (Figure 1). The binding energy was found to be -8.1 kcal/mol (Table 1). The binding energy and the formation of three conventional hydrogen bonding with the crystal structure implied that the ligand could bind to the TEN domain and remain inside the binding pocket.

The investigated antiviral drugs interacted to the TEN domain of TERT (2B2A) with at least two conventional hydrogen bonds and at least three more other types of interactions (Table 1, Figure 1, Figure S1). Some of the drugs had better binding potential than the reference TERT inhibitor, BIBR1532. In this regard, Doravirine, Etravirine, and Rilpivirine had binding energy of -8.7 kcal/mol, -8.9 kcal/mol, and -9.0 kcal/mol, respectively. The binding energy of these drugs was lower than that of BIBR1532 (-8.1 kcal/mol). Therefore, these drugs are expected to have higher binding affinity towards the TEN domain (2B2A). Doravirine and Etravirine had higher number of conventional hydrogen bonds than BIBR1532. Doravirine had five more other types of interactions than the reference. Similarly, Etravirine had two more other types of interactions. On the other hand, Rilpivirine had similar number of conventional hydrogen bonds (3) and other types of interactions (4) with the reference (Table 1, Figure 1). Hence, Doravirine and Etravirine are expected to have higher binding affinity and strength than the reference to the TEN domain. Together with this, Rilpivirine is expected to show higher binding affinity than the reference. From the docking study, it is possible to infer that Doravirine, Etravirine, and Rilpivirine would exhibit higher binding potential to the TEN domain in relative to BIBR1532 as well as the other antiviral drugs.

Table 1. Binding residues of the antiviral drugs and BIBR1532 to the TEN domain of TERT

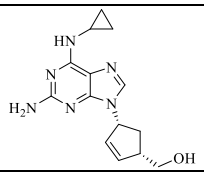
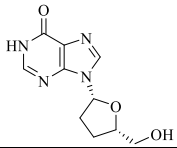
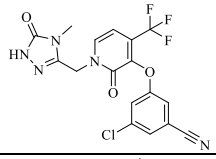
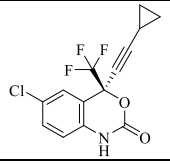
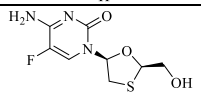
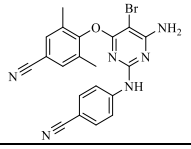
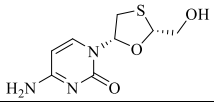
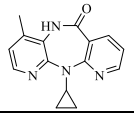
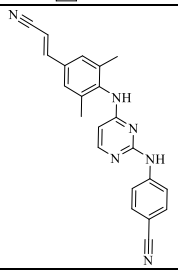
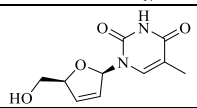
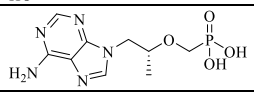
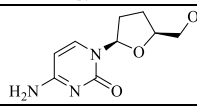
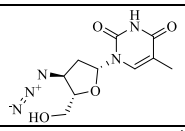
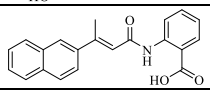
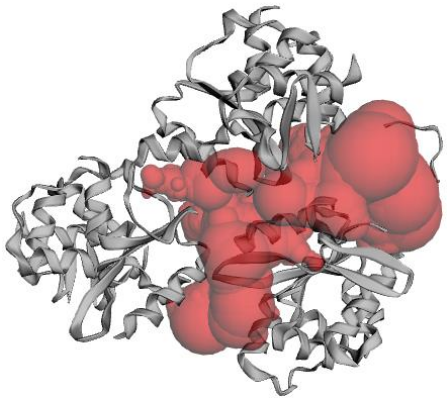
Ligands	Structure	Classification	Binding energy (kcal/mol)	Conventional hydrogen bonding residues	Other interaction residues
Abacavir		NRTI	-6.1	Asp154, Gln176, Tyr177	Glu68(2)[a], Leu71[b], Asp154[c], Val172(2)[b]
Didanosine		NRTI	-6.6	Asp19, Gln163	Leu14[b], Arg16(2)[a], Ala29[b], Lys31[b], Glu162[c]
Doravirine		NNRTI	-8.7	Leu174, Asn175, Trp187, Asn191	Leu20[b], Val23[b], Phe158[b], Leu174[b], Leu174[d], Asn175(2)[c], Phe178[b], Asn191[a]
Efavirenz		NNRTI	-6.1	Thr15, Asn175	Met13[b], Val23[b], Phe158[b], Leu174[b] Asn175[c], Asn191[e], Asn191[a]
Emtricitabine		NRTI	-5.5	Leu14, Arg16	Thr15[c], Arg16[a], Asp19[e], Ala29[b], Lys31[b], Lys31[a]
Etravirine		NNRTI	-8.9	Met13(2), Arg16, Asn70	Val23[b], Lys31[a], Leu174[b], Phe178[b], Asn191(2)[a]
Lamivudine		NRTI	-5.9	Gln69, Glu162	Ala29[b], Ala29[d]
Nevirapine		NNRTI	-6.3	Leu174, Asn175	Gln168[c],Cys173[f], Leu174(2)[b]
Rilpivirine		NNRTI	-9.0	Leu14, Thr15, Gln168	Thr15[d], Lys31[a], Leu174[b], Asn191[a]
Stavudine		NRTI	-6.6	Gln69, Glu162, Gln163	Leu14[b], Ser28[g], Ala29[d]
Tenofovir		NRTI	-5.8	Asn135(2), Tyr177	Ser133[c], Lys160[c]
Zalcitabine		NRTI	-6.2	Arg16(2), Gln69	Leu14[b], Arg16[a], Ala29(2)[b], Asn164[c]

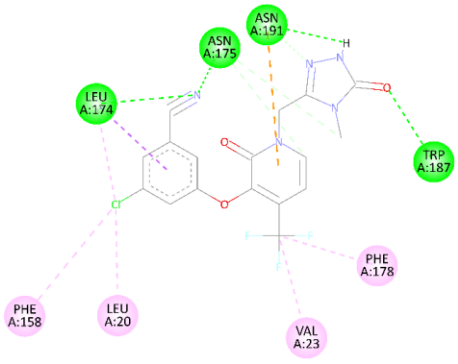
Table 1 (*continue*). Binding residues of the antiviral drugs and BIBR1532 to the TEN domain of TERT

Ligands	Structure	Classification	Binding energy (kcal/mol)	Conventional hydrogen bonding residues	Other interaction residues
Zidovudine		NRTI	-5.3	Gln91, Val119(2)	Asp95[a], Tyr118[g]
BIBR1532			-8.1	Arg16(2), Ser28	Ser28[g], Ala29[d], Lys31[b], Asn191[a]

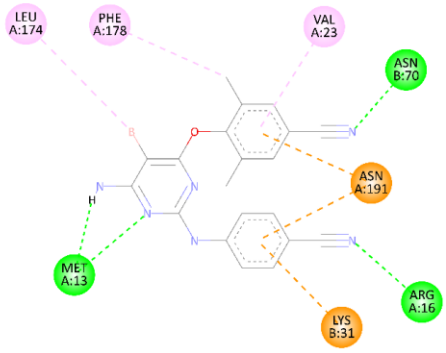
[a]pi-ion, [b]alkyl/pi-alkyl, [c]carbon-hydrogen bond, [d]pi-sigma, [e]halogen, [f]pi-sulfur, [g]pi-pi



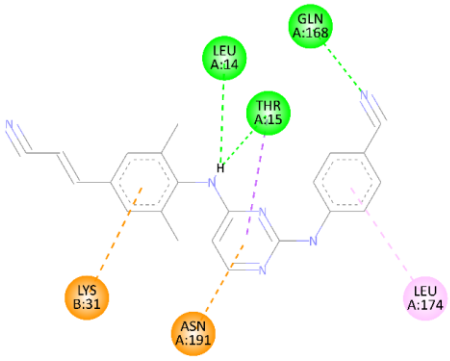
Predicted binding pocket



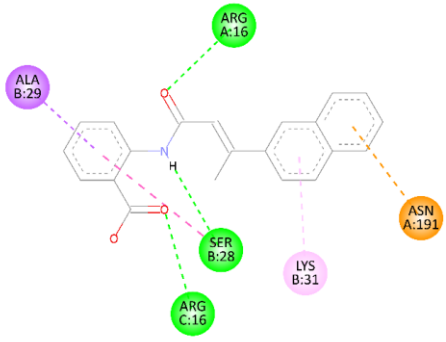
Doravirine



Etravirine



Rilpivirine



BIBR1532

Figure 1. Binding pocket of the target protein and binding profiles of Doravirine, Etravirine and Rilpivirine and BIBR1532 with the TEN domain of TERT (2B2A)

In the docking analysis, the antiviral drugs with the highest binding potential had common interaction residues with the BIBR1532. Doravirine had common interaction points at Asn191 amino acid residue. Etravirine had also common interaction points at Arg16, Lys31, and Asn191 residues. Similarly, Rilpivirine had common interaction points at Lys31 and Asn191 residues. The interaction of the antiviral drugs and the reference ligand implicated that the interaction of the compounds with Asn191 residue played a role for achieving effective binding. A previous computational study revealed that some catechin derivatives had interactions through Gln168 residue as observed in the binding of Rilpivirine in this study. The same study reported that the interaction through Gln162 residue was essential for effective binding of catechins to the TEN domain [25]. In this study, Didanosine, Lamivudine and Stavudine had interactions through this residue but the binding affinity and/or strength of these compounds was less than the three highest binding compounds. Conventional hydrogen bonds were formed between Gln162 and the hydroxyl group of the catechins. Similarly, conventional hydrogen bonds were formed between Gln162 and hydrogen bond donor groups (hydroxyl, amine) of the highest binding compounds in this study.

Structure-activity relation analysis of Doravirine, Etravirine and Rilpivirine revealed that the cyanide (-C≡N) functional group in their structures contributed for effective binding of these drugs to the TEN domain. Two conventional hydrogen bonds of Doravirine and Etravirine were formed between the cyanide functional group and the amino acid residues. Similarly, a conventional hydrogen bond was formed between this functional group of Rilpivirine and the TEN domain (Figure 1). Conventional hydrogen bonds were formed between the amino group of the compounds and various amino acid residues of the target. In this regard, Etravirine had one and Rilpivirine had two conventional hydrogen bonds through their amino group (Figure 1). Similarly, conventional hydrogen bonding was formed with the hydrogen on the nitrogen of the triazole ring for Doravirine and the nitrogen of the pyrimidine ring for Etravirine.

Earlier research performed by scientists has shown that Zidovudine inhibits the action of telomerase and induces the shortening of telomeres. The findings from our molecular docking and molecular dynamics investigation demonstrated that the binding potentials of Doravirine, Etravirine, and Rilpivirine, which are non-nucleoside reverse transcriptase enzyme inhibitors, were higher with binding energies of -8.7 kcal/mol, -8.9 kcal/mol, and -9.0 kcal/mol, respectively. The binding potential of the nucleoside reverse transcriptase enzyme inhibitor Zidovudine was found to be less as its binding energy was higher (-5.3 kcal/mol).

Molecular Dynamics Simulation

The molecular docking study demonstrated that some of the antiviral drugs would have better binding affinity than the reference TERT inhibitor. The stabilities of the complexes formed between the TEN domain and these drugs (Doravirine, Etravirine and Rilpivirine) were investigated by performing MD simulation analysis. To this end, RMSD of the protein, RMSD of the ligands, RMSF, and Rg plots were drawn. A general notion about the effect of ligand binding to the protein's stability was measured by drawing the changes in the RMSD value of the backbone structure. The state of remaining inside the binding pocket of the protein for the ligands during the simulation period was evaluated by using the ligand RMSD value [26,27].

The overall stability of the backbone protein for the compound bearing structures was lower than the unbound structure. The unbound protein structure had the lowest RMSD value after the 18 ns and gave the least RMSD variation during the simulation period (Figure 2). Etravirine bearing complex had lower RMSD value than the other two compound bearing structures. However, it had sharp rises and declines in some time intervals. Doravirine bearing complex had similar RMSD value with Etravirine bearing complex after the 150 ns time but a higher RMSD value till then. Together with this, its RMSD value variation was not as sharp as that of Etravirine bearing complex. Rilpivirine bearing complex had the highest RMSD value and variations during the simulation period. Especially, in the 20-100 ns time interval, it gave high rises and falls that implicated instability for this complex (Figure 2).

The state of the compounds to remain inside the binding pocket of the target was evaluated by drawing the RMSD plot of the compounds in relative to the protein structure. The ligand RMSD plot demonstrated that Doravirine could remain inside the binding pocket during the simulation period. In

general, the RMSD value of Doravirine was below 0.5 nm (Figure 2). Etravirine had above 0.5 nm RMSD value with some exception time intervals. In addition to this, it had a sharp RMSD value rise in the 132-139 ns time interval. The compound might fly out of the binding pocket in this interval. Rilpivirine had generally high RMSD value that went up to 2.5 nm and also high variations throughout the simulation period. The high degree of changes in the RMSD value implicated that the compound would move in and then out of the binding pocket. Therefore, this would hinder its binding potential to form a stable complex with the protein. The RMSD plots demonstrated that Doravirine had the highest potential to remain inside the binding pocket and thus retain its binding potential to form a stable protein-compound complex.

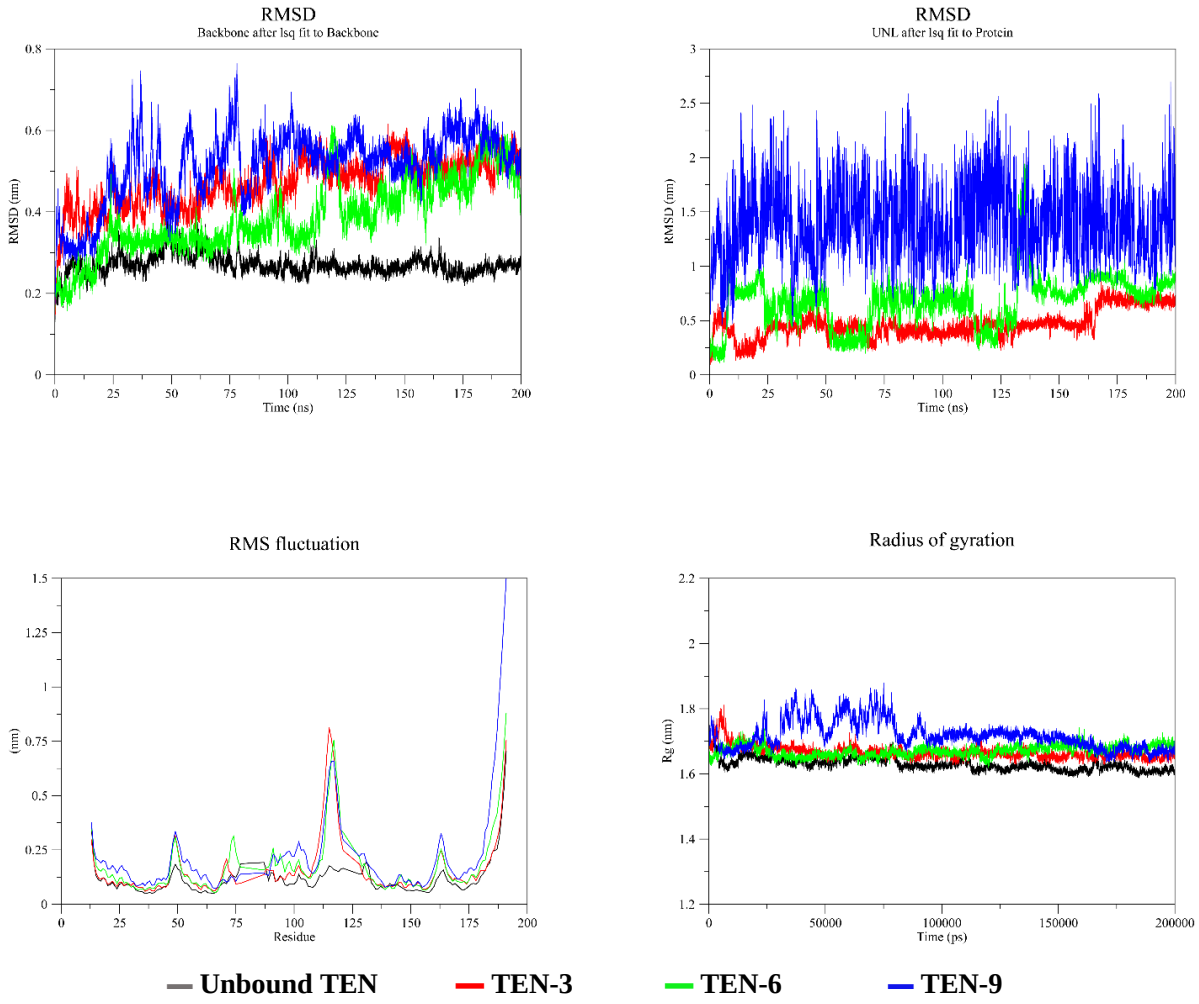


Figure 2. RMSD, RMSF, and Rg plots from the MD simulation trajectories

The per-residue fluctuations of the unbound protein and complex structures were evaluated by the RMSF plot. In general, all of the evaluated structures had similar RMSF plots throughout the simulation period. A significant RMSF value change was detected in the C-terminal for all of the structures. Similarly, all of the structures except the unbound protein had significant RMSF value changes in the 110-130 amino acid intervals (Figure 2). The effect of compound binding on the compactness of the structures was evaluated via Rg plot [28]. The compactness of the protein structure has decreased by the binding of Rilpivirine on it. Especially, in the 20-95 ns time interval, the complex was less compact than the other complexes and the unbound protein. The effect of the binding of Doravirine and Etravirine on the firmness of the protein was similar as they gave similar Rg values

during the simulation period. Furthermore, the change in the firmness of the protein structure by the binding of Doravirine and Etravirine was insignificant.

Conclusion

Antiviral drugs were evaluated for their potential to inhibit cancer by targeting the TEN domain of TERT. Doravirine, Etravirine and Rilpivirine showed higher binding affinity to the TEN domain compared to BIBR1532, the reference TERT inhibitor, according to the results of the molecular docking study. Molecular dynamics simulation showed that Doravirine formed a stable combination with the protein. Thus, Doravirine was able to persist in the binding pocket of the TEN domain and maintain its binding ability throughout the duration of the simulation. In short, Doravirine was able to bind to the TEN domain and inhibit it. The potential of Doravirine as an anticancer agent by inhibiting the TEN domain of TERT should be confirmed by further *in vitro* and *in vivo* investigations based on computational studies. Based on this investigation, we will design the structures of telomerase enzyme inhibitor compounds to be developed, using the structures of these 3 drugs as a reference.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflicts of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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