

Studying the Mechanism Underlying the Inhibitory Activity of (S)- β Citronellol against HIV-1 Reverse Transcriptase

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Abstract: *Cymbopogon nardus* essential oil (EO) inhibits human immunodeficiency virus reverse transcriptase 1 (HIV-1 RT) activity dose-dependently and in a specific manner. This inhibitory activity is induced by (S)- β -citronellol in *C. nardus* EO. This study investigated how (S)- β -citronellol inhibits HIV-1 RT in vitro using an HIV-1 RT colorimetric enzyme immunoassay and in silico using a molecular operating environment docking simulation computer program. (S)- β -citronellol bound to the non-nucleosides inhibitors binding pocket (NNIBP) of the wild-type (WT) HIV-1 RT in a similar way as bind existing non-nucleoside HIV-1 RT inhibitor acquired immunodeficiency syndrome drugs and lysine located way as do at position 102 in the DNA sequence of the WT HIV-1 RT contained may be directly or indirectly involved in (S)- β -citronellol binding to the NNIBP.

Keywords: (S)- β -citronellol, human immunodeficiency virus type 1 reverse transcriptase, molecular operating environment, non-nucleoside inhibitors binding pocket, non-nucleoside reverse transcriptase inhibitor

Abbreviations: AIDS: Acquired immunodeficiency syndrome; *C. nardus*: *Cymbopogon nardus*; Dig-11-dUTP: Digoxigenin-11-deoxyuridine triphosphate; dTTP: Deoxythymidine triphosphate; EDTA: Ethylenediaminetetraacetic acid; EFV: Efavirenz; EO: Essential oil; EtOH: Ethanol; GC/MS: Gas chromatography-mass spectrometry; HIV-1: Human immunodeficiency virus type 1; MOE: Molecular operating environment; NNIBP: Non-nucleoside inhibitors binding pocket; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PDB: Protein data bank; RT: Reverse transcriptase; Tris: Tris (hydroxymethyl) aminomethane; WT: Wild type

1. INTRODUCTION

Human immunodeficiency virus 1 (HIV-1)-positive cases were first reported in the United States in 1981 among gay males and injecting drug users, and the number of patients has constantly been increasing worldwide. Therefore, acquired immunodeficiency syndrome (AIDS) is one of the most severe public health threats in history^{1,2,3,4}. Recently, the trend has remained high in some regions of the world⁵. In 2019, the World Health Organization estimated that over 40 million people were infected worldwide and that the incidence of new infections has reached approximately 50,000 cases per year, especially among certain socio-economic groups⁶. The primary HIV-1 transmission routes include sexual contact, blood transmission, and mother-to-child transmission. Approximately 10 years after infection, various clinical symptoms such as fever, diarrhea, health discomforts, swollen lymph nodes, and weight loss appear. Further progression leads to AIDS. In addition, opportunistic infections, including *Pneumocystis jirovecii* pneumonia and esophageal candidiasis, tumors such as Kaposi's sarcoma, and neurological symptoms such as dementia are frequent.

AIDS symptoms are primarily caused by decreased cell-mediated immunity owing to HIV-1 infection. The decrease in the efficiency of cell-mediated immunity is a clinical factor directly related to the prognosis, and the majority of AIDS-related deaths are caused by a weakened immune system induced by infections and opportunistic diseases. Therefore, preventing infection from the onset of AIDS through early detection and adequate treatment is essential to improve the life quality of patients living with AIDS.

Approximately 52 years ago, Temin and Baltimore discovered that HIV-1 has an RNA-dependent DNA polymerase enzyme called HIV-1 reverse transcriptase (RT) that humans do not possess and proved that the enzymatic activity of RT is indispensable for HIV-1 replication^{7,8}. Following to the infection, the HIV-1 RT enzyme copies the viral genome into a ds DNA capable of being integrated into the host cell chromosome DNA with a large amount of natural information of endogenous retroviruses. Therefore, the onset of AIDS can be efficiently prevented if a drug that inhibits this enzyme can be developed, which would not be toxic to humans and could be administered over a long period. Because RT is a critical step in the life cycle of HIV-1, many anti-AIDS drug researchers have focused on developing HIV-1 RT inhibitor (HIV-1 RTI) drugs designed to target and inhibit RT.

Two groups of RTIs exist: nucleoside RT inhibitors (NRTIs) designed to inhibit RT activity competitively and non-nucleoside RT inhibitors (NNRTIs) designed to inhibit RT activity non-competitively. The NNRTIs group includes first-generation NNRTIs like efavirenz (EFV) and second-generation NNRTIs like etravirine. Highly active antiretroviral therapy (HAART) is a combination of three or more antiretroviral drugs, which typically includes an NNRTI or protease inhibitor in combination with at least two NRTIs to minimize the risk of the virus developing resistance to all three drugs simultaneously. HAART has therefore become the mainstay treatment in individuals infected with HIV-1. Furthermore, HAART has substantially reduced the mortality and morbidity of patients with AIDS⁹.

However, using anti-HIV drugs, especially HIV-1 RT inhibitors, still encounters many problems, such as toxicity and drug resistance due to long-term use^{10,11,12}. Moreover, HAART has adverse events and resulted in the development of more complex and drug-resistant viruses¹³. The occasional mutation of amino acids in the enzymatic HIV-1 domain targeted by antiviral drugs, especially NNRTIs, skillfully eliminates the antiviral drugs or impedes their binding with the enzyme. The virus then maintains its original enzymatic activities and replication. Adverse effects, drug toxicities, resistance, the non-availability of adequate AIDS drugs, and the treatment cost, mostly in developing countries with limited resources, oblige HIV-1 RT inhibitors researchers to focus on the global challenge of discovering new and natural sources of RT inhibitors¹⁴. Therefore, current AIDS drug researches focus on clarifying and classifying the mechanisms leading to HIV-1 resistance to NNRTIs, considering their molecular mechanisms and pathways for developing novel natural antiviral agents¹⁵. Several secondary metabolites from natural products, such as alkaloids, flavonoids, sulfated polysaccharides, coumarins, and triterpenes, inhibit viral replication steps such as reverse transcription¹⁶. Bioactive flavonoids (myricetin, quercetin, and pinocembrin), derived from plant tissues, have potent anti-HIV activity in a non-toxic concentration range²³. Some plant extracts (*Malva sylvestris*) and fractions presented anti-inflammatory and anti-osteoclastogenic antioxidant activities and strong immunity-enhancing activities at very low concentrations¹⁷. Such plant extracts can enhance the activity of synthetic compounds and serve as an AIDS drug with anti-HIV-1 RT agents. Therefore, African communities have developed many effective natural medicines using traditional therapeutic plants¹⁸. These studies and trials indicated that screening anti-HIV-RT inhibitor compounds derived from natural products can lead to new drug discovery. However, studies elucidating how natural products, such as essential oils (EOs) and honey constituents, can be used as potential HIV-1 RT inhibitors and overcome some HIV-1 RT inhibitor-related problems are scarce.

In the Republic of Benin (West Africa), many African traditional medicine plants (ATMPs) have been used to treat infections caused by hepatitis viruses and HIV-1. *Cymbopogon nardus* (*C. nardus*), one of these ATMPs, contains EO and efficiently treats patients with HIV-1. In our previous study, *C. nardus* EO inhibited HIV-1 RT and (S)- β -citronellol, the active compound of *C. nardus* EO, also inhibited HIV-1 RT in dose-dependently and in a specific manner. However, the mechanism of action of (S)- β -citronellol is still unknown¹⁹. Therefore, we elucidated the inhibitory mechanism using HIV-1 RT inhibitory assay colorimetric *in vitro* experiments in this study. Furthermore, we used the molecular modeling simulation software, molecular operating environment (MOE), to observe the complex formed by RT bound to (S)- β -citronellol inhibitor during inhibition through *in silico* experiments.

2. MATERIALS AND METHODS

2.1. Evaluation of HIV-1 RT Inhibitory Effects

The HIV-1 RT inhibitory effects of EO were quantitatively determined using a colorimetric enzyme

immunoassay that incorporated DIG- and biotin-labeled dUTPs into native DNA. The assay was performed with an RT assay colorimetric kit (Roche, Basel, Switzerland), following the manufacturer's instructions, with slight modifications. Briefly, 60 μ L of the reaction mixture containing final concentrations of 46 mM Tris-HCl, 266 mM KCl, 27.5 mM MgCl₂, 9.2 mM dithiothreitol (Roche), 10 mM biotin-16-dUTP/digoxigenin deoxyuridine triphosphate (DIG-dUTP; Roche), 2.2 μ g/mL PolyA:oligo (dT)₁₅ template/primer hybrid, 1.7 ng/mL HIV-1 RT (provided by Kumamoto University of Pharmacy in Japan), and various concentrations of the sample (0.1–10 mg/mL) was incubated at 37°C for approximately 15 h. The hexanucleotide mix (10 \times concentration) was purchased from Roche. The reaction mixture was then transferred to the surface of streptavidin-coated enzyme-linked immunosorbent assay (ELISA) plate wells. After removing any unbound substances by washing, an antibody directed against digoxigenin conjugated to peroxidase (anti-digoxigenin-peroxidase) was added to bind to DIG-11-dTTP. After another wash, 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was added. The absorbance (OD₄₀₅ nm) of the color that developed in the presence of ABTS was evaluated at OD₄₀₅ nm using an ELISA microplate reader (SpectraMax 340PC). A reaction solution that replaced the inhibitor with a dilution buffer was used as a control. A sample without RT solution was used as the negative control, and a sample with 2 mM ddTTP (99% purity by high-performance liquid chromatography; Amsterdam Biosciences, Amsterdam, Netherlands) was used as the positive control. (S)- β -citronellol (>95% purity by gas chromatography; Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) was dissolved in ethanol to prepare a dilution series of 0.1–10 mg/mL. The final ethanol concentration in the solutions was <1%, which did not affect the assay results. The inhibitory effects of RT inhibitors were calculated as the inhibition percentage relative to the control, as follows:

Control relative percentage of inhibition = $100 - [(Sample\ OD_{405}\ nm - Negative\ control\ OD_{405}\ nm) / (Control\ OD_{405}\ nm - Negative\ control\ OD_{405}\ nm)] \times 100$

2.2. Docking Simulation of (S)- β -citronellol on two HIV-1 RTs Types

The inhibitory activities of (S)- β -citronellol inhibitory activity against WT HIV-1 RT and clinical HIV-1 RT enzymes were compared. The operating procedure for the docking simulation of (S)- β -citronellol was performed according to the MOE simulation program according to the manual (Morsis, Japan). MOE docking simulation was performed using downloaded WT HIV-1 RT structures with lysine at position 102 (Protein data bank (PDB) code: 1IKW, 3M8Q, 2RF2), clinical HIV-1 RT structures with glutamine at position 102 (PDB code: 4ZHR and 5XN1), the x-ray crystal structure of efavirenz NNRTI, and (S)- β -citronellol chemical structure. The (S)- β -citronellol chemical structure was applied to the HIV-1 RT structure reference 1IKW with 1,000, 1,500, 3,000, and 10,000 searches, to the HIV-1 RT structure reference 3M8Q with 30,000 searches, and the HIV-1 RT structure reference 2RF2 with 15,000 searches. Similarly, the (S)- β -citronellol chemical structure was applied to the clinical HIV-1 RT with 1,000, 1,500, 3,000, and 10,000 searches for 4ZHR and 30,000 searches for 5XN1.

2.3. Statistical Analysis

The results were analyzed using the Student's t-test. Differences were considered significant at $p < 0.05$

3. RESULTS

3.1. Comparison of the (S)- β -citronellol Inhibitory Activity against Two HIV-1 RTs

Because (S)- β -citronellol showed inhibitory effects on commercially available WT RT, we investigated its inhibitory effect against HIV-1 RT derived from patients with HIV-1 (clinical HIV-1 RT). Figure 1 compares the inhibitory effects of (S)- β -citronellol against WT HIV-1 RT and clinical HIV-1 RT (Figs. 1A and 1B). *In vitro* immunoassay experiments confirmed that (S)- β -citronellol exhibited dose-dependent inhibitory activity against clinical HIV-1 RT enzymes. However, the inhibition rate was very weak (Fig. 1B).

Moreover, when 1 mg/mL (S)- β -citronellol was applied to the WT HIV-RT enzyme, the WT RT activity was approximately 40% inhibited, and 10 mg/mL (S)- β -citronellol solution inhibited it at approximately 65%. In contrast, when the same (S)- β -citronellol concentrations were applied to the clinical HIV-RT enzyme, the inhibition rate shown by 3 mg/mL of (S)- β -citronellol against the activity of this RT was 7.3%. Even 10 mg/mL (S)- β -citronellol could only inhibit the activity of the clinical HIV-1 RT at approximately 23.4% compared to the control. These results showed that WT

HIV-1 RT was approximately 42% more strongly inhibited when 10 mg/mL (S)- β -citronellol was applied (Figs.1A and 1B).

We then examined the DNA sequences of the clinical HIV-1 RT and that of the WT HIV-1 RT enzymes to determine what might be responsible for the difference in the inhibition rate of these two HIV-1 RTs. The clinical HIV-1 RT enzyme had glutamine (Gln) at position 102, whereas the WT HIV-1 RT enzyme had lysine (Lys) at the same position in their DNA sequences (Fig.2).

3.2. Molecular Modeling Simulation of (S)- β -citronellol on HIV-1 RTs

Based on the evident differences in amino acids noticed in the DNA sequences of these two RTs, we used the MOE docking simulation program to observe the complex formed by (S)- β -citronellol and each of these RTs. The x-ray crystal structure of EFV was used to investigate whether (S)- β -citronellol binds to WT RT or clinical RT through the NNIBP used by NNRTIs for binding to RT. The results of the molecular modeling simulation using the structure of the WT HIV-1 RT enzyme (Lys102) according to PDB references 1IKW, 3M8Q, and 2RF2 are shown in Figures 3A, B, C, and D.

The diagram of the interaction between (S)- β -citronellol and HIV-1 RT enzyme in the 2FR2 score top 10 poses show the positioning of (S)- β -citronellol in the NNIBP, indicating that (S)- β -citronellol is positioned very close to Lys101, Tyr181, and Tyr188 (Figs.3B, C, and D). The results of the MOE docking simulation of (S)- β -citronellol on WT RT using the 1IKW and 3M8Q references were similar to those using the reference 2FR2 (Figs.3B, C, and D). The binding modes of score 4ZHR and score 5XN1 top 10 poses are shown in Figure 4 for the MOE simulation of (S)- β -citronellol on the clinical HIV-1 RT structure (with Gln102). Enlarging the 2D view of NNIBP showed no binding between (S)- β -citronellol and the clinical HIV-1 RT NNIBP (Figs. 4B and C).

4. DISCUSSION

The inhibitory activity of (S)- β -citronellol against WT HIV-1 RT was stronger than that against clinical HIV-1 RT. Our *in vitro* RT colorimetric assay showed that the inhibitory rate of (S)- β -citronellol against the clinical HIV-1 RT enzyme was approximately 50% lower than the inhibitory rate of the same (S)- β -citronellol concentration against the WT HIV-1 RT enzyme. By comparing the DNA sequences of the clinical HIV-1 RT enzyme with that of the WT HIV-1 RT enzyme, adenine was identified at position 306 of the DNA sequence of the WT RT, whereas cytosine was identified at the same position of the DNA sequence of the clinical RT. Because of this change in position 304 of the DNA sequences of these two HIV-1 RTs, the original AAG codon corresponding to Lys in the WT RT DNA sequence was changed to CAG, corresponding to Gln in that of the clinical RT. These changes led to the occurrence of the Lys102Gln mutation.

Amino acid mutations in the DNA sequence of RT may have substantial consequences, especially on the inhibitory activity of NNRTIs against the HIV-1 RT enzyme²⁰. However, no study has reported that the Lys102 mutation is responsible for HIV-1 RT resistance to NNRTIs. In some mutant RT forms, Van der Waals interactions were observed between NNRTIs and Lys102, but Lys102 was not indicated as the amino acid used by NNRTIs for binding to NNIBP²². Furthermore, Leu100, Lys103, Val106, Tyr181, and Tyr188 are involved in the binding of inhibitors to NNIBP, and slight mutations in Lys103 surrounding amino acids, especially Lys102, distort and considerably affect NNRTI binding to the HIV-1 RT NNIBP site²¹. This finding led us to hypothesize that the mutation at position 102 of the clinical RT sequence may have negatively affected the binding of (S)- β -citronellol to the NNIBP, decreasing the incorporation rate of dNTPs in cDNA.

The docking simulation of (S)- β -citronellol on the WT RT (with Lys102) shows that the totality of the (S)- β -citronellol molecules seemed to be densely gathered in NNIBP. In contrast, the docking simulation of (S)- β -citronellol on clinical RT (with Gln102) showed that just a few (S)- β -citronellol molecules seemed to be gathered in NNIBP while the remaining molecules were scattered in other receptors. Furthermore, molecular modeling simulations showed no results, suggesting that (S)- β -citronellol primarily binds to the NNIBP of the clinical HIV-1 RT enzyme. Enlarging the 2D view of the NNIBP of clinical RT showed no bond between (S)- β -citronellol and clinical HIV-1 RT NNIBP. Therefore, we concluded that the Lys102 Gln mutation at position 102 of the clinical RT DNA sequence may have negatively affected the hydrogen binding of (S)- β -citronellol to Lys101. For this reason, the rate of the inhibitory effects of (S)- β -citronellol against clinical HIV-1 RT was approximately 50% weaker than that of the inhibitory effects of (S)- β -citronellol against WT HIV1 RT. Therefore, Lys102 might be directly or indirectly involved in binding (S)- β -citronellol to NNIBP of the WT HIV-1 RT enzyme.

The primary aim of this study was to elucidate the mechanism of the inhibitory effects of (S)- β -citronellol against the HIV-1 RT enzyme. However, to verify whether (S)- β -citronellol binds or not to the NNIBPs used by existing NNRTIs, it may be necessary to generate recombinant HIV-1 RT enzymes with Leu100, Lys103, Val106, Tyr181, and Tyr188 mutations, which are critical amino acids for the binding of NNRTIs to the NNIBP. Furthermore, investigating the inhibitory activity of (S)- β -citronellol on these generated HIV-1 RT enzymes is also necessary.

5. CONCLUSION

Enzyme inhibition experiments were performed using WT HIV-1 RT and clinical HIV-1 RT. The (S)- β -citronellol concentration needed to inhibit RT up to 75% of the control, was 0.1 mg/mL when WT HIV-1 RT was applied and 10 mg/mL when clinical HIV-1 RT was applied. This result showed that the inhibitory effect of (S)- β -citronellol against clinical HIV-1 RT was approximately 100 times weaker than in clinical RT. Adenine at position 304 was mutated to cytosine in clinical RT, substituting the amino acid at position 102 from Lys to Gln. Furthermore, (S)- β -citronellol binds to NNIBP of WT HIV-1 RT but not to that of clinical RT, possibly because the mutation at amino acid 102 distorts NNIBP and prevents (S)- β -citronellol from binding to NNIBP. Consequently, the inhibitory activity against clinical RT was reduced to approximately 1/100 in enzyme inhibition experiments. Our study indicates that the mechanism of the action of (S)- β -citronellol against HIV-1 RT likely involves binding (S)- β -citronellol to the NNIBP of the HIV-1 RT enzyme containing Lys102 which may be important in this inhibitory mechanism.

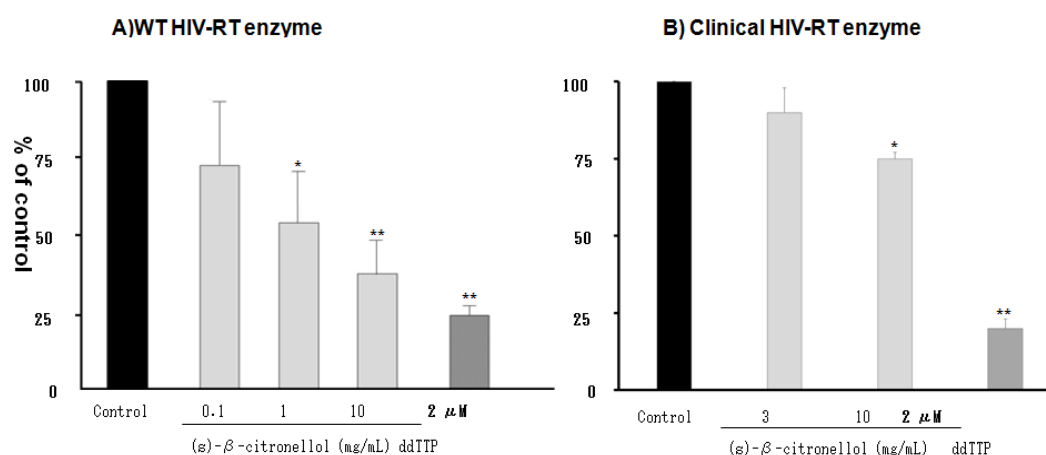


Fig1. Comparative experiments of the inhibitory activity of (S)- β -citronellol against the RTs from two strains of HIV-1. Student's t-test. * $p < 0.05$ vs the appropriate control, ** $p < 0.01$ vs the appropriate control.

1'	CCCATTAGTC	CTATTGAGAC	TGTACCAGTA	AAATTAAGC	CAGGAATGGA	TGGCCAAAA
	***** *	*****	*****	*****	*****	*****
	CCCATTAGCC	CTATTGAGAC	TGTACCAGTA	AAATTAAGC	CAGGAATGGA	TGGCCAAAA
61'	GTTAAACAAT	GGCCATTGAC	AGAAGAAAA	ATAAAAGCAT	TAGTAGAAAT	TTGTACAGAA
	*****	*****	*****	*****	*****	*****
	GTTAAACAAT	GGCCATTGAC	AGAAGAAAA	ATAAAAGCAT	TAGTAGAAAT	TTGTACAGAG
121'	ATGAAAAAGG	AAGGAAAAAT	TTCAAAAAAT	GGGCCTGAAA	ATCCATACAA	TACTCCAGTA
	*****	*****	*****	*****	*****	*****
	ATGAAAAAGG	AAGGAAAAAT	TTCAAAAAAT	GGGCCTGAAA	ATCCATACAA	TACTCCAGTA
181'	TTTGCCATAA	AGAAAAAGA	CAGTACTAAA	TGGAGAAAAT	TAGTAGATTT	CAGAGAACTT
	*****	*****	*****	*****	*****	*****
	TTTGCCATAA	AGAAAAAGA	CAGTACTAAA	TGGAGAAAAT	TAGTAGATTT	CAGAGAACTT
241'	AATAAGAGAA	CTCAAGATTT	CTGGGAAGTT	CAATTAGGAA	TACCACATCC	TGCAGGGTTA
	*****	***** **	*****	*****	*****	*****
	AATAAGAGAA	CTCAAGACTT	CTGGGAAGTT	CAATTAGGAA	TACCACATCC	CGCAGGGTTA
301'	AAACAGAAAA	AATCAGTAAC	AGTACTGGAT	GTGGGCGATG	CATATTTTTC	AGTTCCCTTA
	*** *****	*****	*****	*****	*****	*****

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AAAAGAAAA AATCAGTAAC AGTACTGGAT GTGGGTGATG CATATTTTTC AGTTC CCTTA
361' GATAAAGACT TCAGGAAGTA TACTGCATTT ACCATACCTA GTATAAACAA TGAGACACCA
***          *****
GATGAAGACT TCAGGAAGTA TACTGCATTT ACCATACCTA GTATAAACAA TGAGACACCA
421' GGGATTAGAT ATCAGTACAA TGTGCTTCCA CAGGGATGGA AAGGATCACC AGCAATATTC
*****
GGGATTAGAT ATCAGTACAA TGTGCTTCCA CAGGGATGGA AAGGATCACC AGCAATATTC
481' CAGTGTAGCA TGACAAAAAT CTTAGAGCCT TTTAGAAAAC AAAATCCAGA CATAGTCATC
**          *****
CAAAGTAGCA TGACAAAAAT CTTAGAGCCT TTTAGAAAAC AAAATCCAGA CATAGTTATC
541' TATCAATACA TGGATGATTT GTATGTAGGA TCTGACTTAG AAATAGGGCA GCATAGAACA
*****
TATCAATACA TGGATGATTT GTATGTAGGA TCTGACTTAG AAATAGGGCA GCATAGAACA
601' AAAATAGAGG AACTGAGACA ACATCTGTTG AGGTGGGGAT TTACCACACC AGACAAAAAA
*****
AAAATAGAGG AGCTGAGACA ACATCTGTTG AGGTGGGGAC TTACCACACC AGACAAAAAA
661' CATCAGAAAG AACCTCCATT CCTTTGGATG GGTATGAAC TCCATCCTGA TAAATGGACA
*****
CATCAGAAAG AACCTCCATT CCTTTGGATG GGTATGAAC TCCATCCTGA TAAATGGACA
721' GTACAGCCTA TAGTGCTGCC AGAAAAGGAC AGCTGGACTG TCAATGACAT ACAGAAATTA
*****
GTACAGCCTA TAGTGCTGCC AGAAAAGGAC AGCTGGACTG TCAATGACAT ACAGAAATTA
781' GTGGGAAAAT TGAATTGGGC AAGTCAGATT TATGCAGGGA TTAAAGTAAG GCAATTATGT
*****
GTGGGAAAAT TGAATTGGGC AAGTCAGATT TATGCAGGGA TTAAAGTAAG GCAATTATGT
841' AAACCTCTTA GGGGAACCAA AGCACTAACA GAAGTAGTAC CACTAACAGA AGAAGCAGAG
*****
AAACCTCTTA GAGGAACCAA AGCACTAACA GAAGTAATAC CACTAACAGA AGAAGCAGAG
901' CTAGAACTGG CAGAAAACAG GGAGATTCTA AAAGAACCGG TACATGGAGT GTATTATGAC
*****
CTAGAACTGG CAGAAAACAG AGAGATTCTA AAAGAACCAG TACATGGAGT GTATTATGAC
961' CCATCAAAAAG ACTTAATAGC AGAAAATACAG AAGCAGGGGC AAGGCCAATG GACATATCAA
*****
CCATCAAAAAG ACTTAATAGC AGAAAATACAG AAGCAGGGGC AAGGCCAATG GACATATCAA
1021' ATTTATCAAG AGCCATTTAA AAATCTGAAA ACAGGAAAAGT ATGCAAGAAT GAAGGGTGCC
*****
ATTTATCAAG AGCCATTTAA AAATCTGAAA ACAGGAAAAT ATGCAAGAAT GAGGGGTGCC
1081' CACACTAATG ATGTGAAACA ATTAACAGAG GCAGTACAAA AAATAGCCAC AGAAAGCATA
*****
CACACTAATG ATGTAAAACA ATTAACAGAG GCAGTGCAAAA AAATAACCAC AGAAAGCATA
1141' GTAATATGGG GAAAGACTCC TAAATTTAAA TTACCCATAC AAAAGGAAAC ATGGGAAGCA
*****
GTAATATGGG GAAAGACTCC TAAATTTAAA CTGCCATAC AAAAGGAAAC ATGGGAAACA
1201' TGGTGGACAG AGTATTGGCA AGCCACCTGG ATTCTTGAGT GGGAGTTTGT CAATACCCCT
*****
TGGTGGACAG AGTATTGGCA AGCCACCTGG ATTCTTGAGT GGGAGTTTGT TAATACCCCT
1261' CCCTTAGTGA AGTTATGGTA CCAGTTAGAG AAAGAACCCA TAATAGGAGC AGAAACTTTC
*****
CCCTTAGTGA AATTATGGTA CCAGTTAGAG AAAGAACCCA TAGTAGGAGC AGAAACTTTC
1321' TATGTAGATG GGCAGCCAA TAGGGAAACT AAATTAGGAA AAGCAGGATA TGTAAGTAC
*****
TATGTAGATG GGCAGCTAA CAGGGAGACT AAATTAGGAA AAGCAGGATA TGTACTAAT

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1381'	AGAGGAAGAC	AAAAAGTTGT	CCCCCTAACG	GACACAACAA	ATCAGAAGAC	TGAGTTACAA
	*****	*****	* *****	*****	*****	*****
	AGAGGAAGAC	AAAAAGTTGT	CACCCTAACT	GACACAACAA	ATCAGAAGAC	TGAGTTACAA
1441'	GCAATTCATC	TAGCTTTGCA	GGATTTCGGGA	TTAGAAGTAA	ACATAGTGAC	AGACTCACAA
	*****	***	*****	*****	*****	***
	GCAATTTATC	TAGCTTTGCA	GGATTTCGGGA	TTAGAAGTAA	ACATAGTAAC	AGACTCACAA
1501'	TATGCATTGG	GAATCATTCA	AGCACAAACCA	GATAAGAGTG	AATCAGAGTT	AGTCAGTCAA
	*****	*	*****	*** * ****	*****	*****
	TATGCATTAG	GAATCATTCA	AGCACAAACCA	GATCAAAGTG	AATCAGAGTT	AGTCAATCAA
1561'	ATAATAGAGC	AGTTAATAAA	AAAGGAAAAA	GTCTACCTGG	CATGGGTACC	AGCACACAAA
	*****	*****	*****	*****	*****	*****
	ATAATAGAGC	AGTTAATAAA	AAAGGAAAAA	GTCTATCTGG	CATGGGTACC	AGCACACAAA
1621'	GGAATTGGAG	GAAATGAACA	AGTAGATAAA	TTGGTCAGTG	CTGGAATCAG	G
	*****	*****	*****	** *****	*****	*
	GGAATTGGAG	GAAATGAACA	AGTAGATAAA	TTAGTCAGTG	CTGGAATCAG	G

Fig2. Comparison of DNA sequences between clinical HIV-1 RT (upper) and wild-type RT (lower, AF033819.3). The letters in red correspond to the mutated 102nd amino acid (lysine mutated to glutamine).

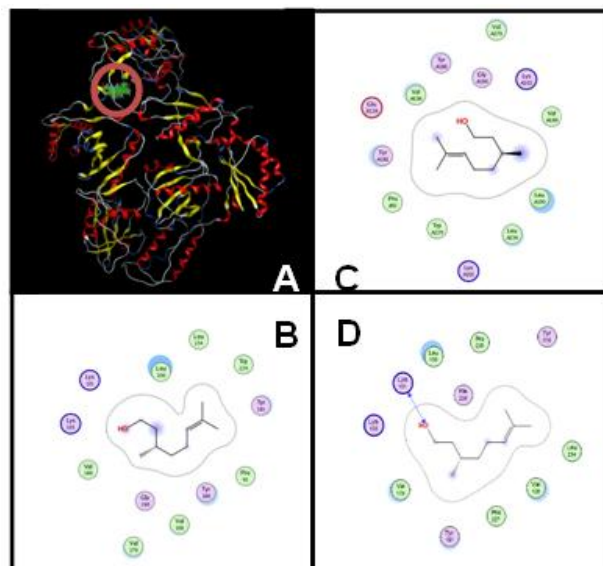


Fig3. Docking simulation of (S)- β -citronellol on the WT RT (Lys.102). Green part: (S)- β -citronellol molecules gathered densely in NNIBP (in pink), as shown in the top 10 poses of score 2FR2. Molecular modeling simulation of the complex formed by (S)- β -citronellol bound to the NNIBP of the WT RT (A). 2D view enlargement of the binding mode in scores 11KW (B), 3M8Q (C), and 2RF2 (D) top poses.

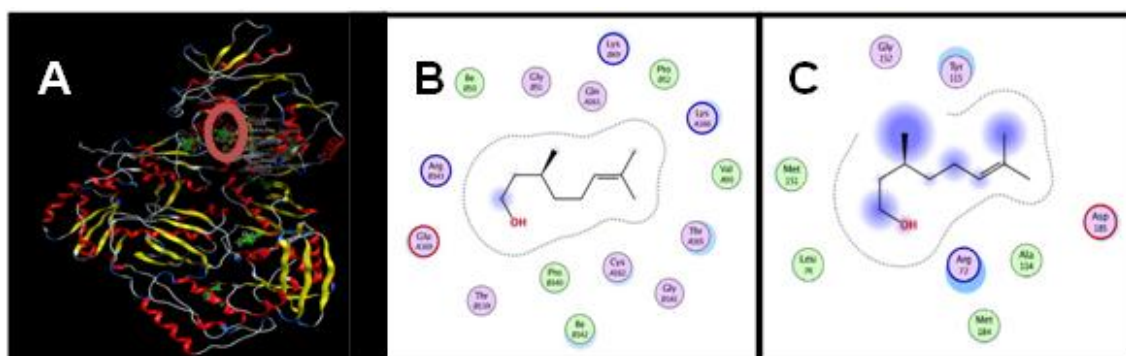


Fig4. Docking simulation of (S)- β -citronellol on the HIV-1 RT enzyme with Gln102 (clinical HIV-1 RT enzyme). Green part: (S)- β -citronellol molecules. NNIBP clinical RT (indicated in red round) in the top 10 poses of scores 4ZHR and 5XN1 (A). 2D view of the molecular modeling simulation of the binding mode in the top 10 poses of score 4ZHR (B). 2D view of the molecular modeling simulation of binding mode in the top 10 poses of score 5XN1 (C).

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