Alternative HIV-1 Non-Nucleoside Reverse Transcriptase Inhibition: Targeting the p51 subunit

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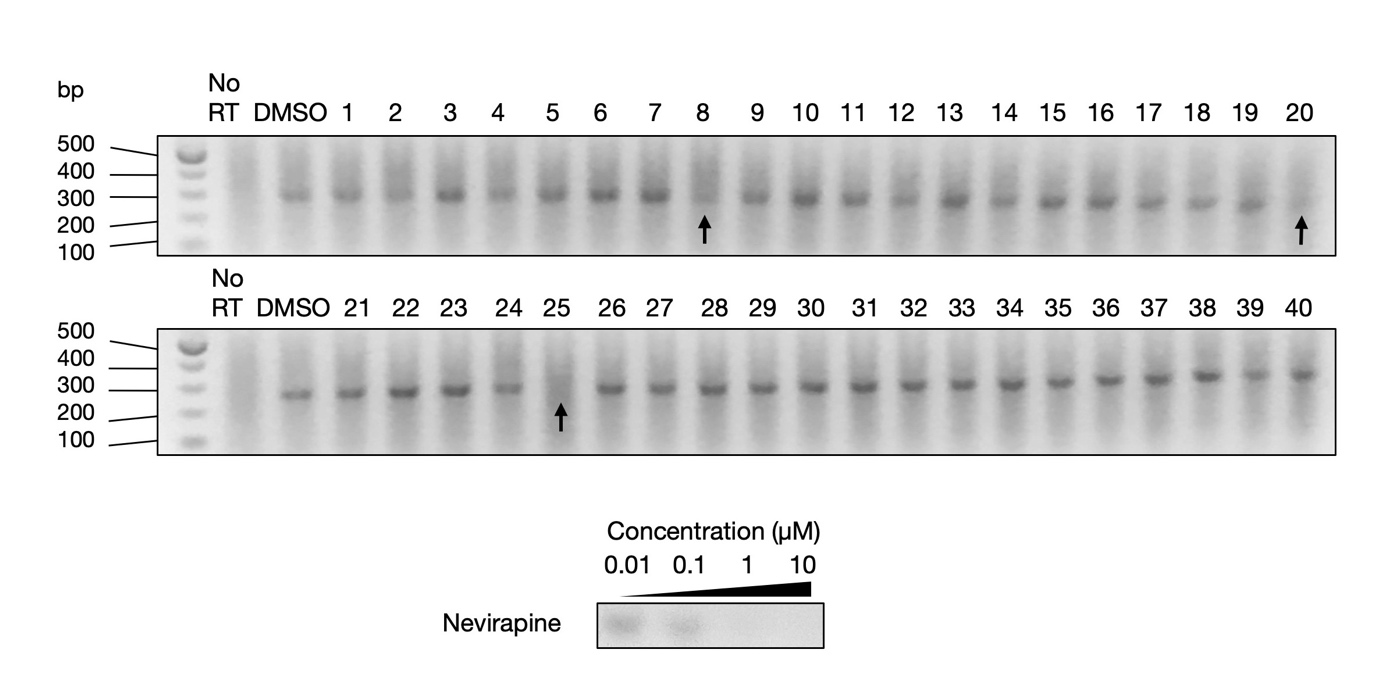
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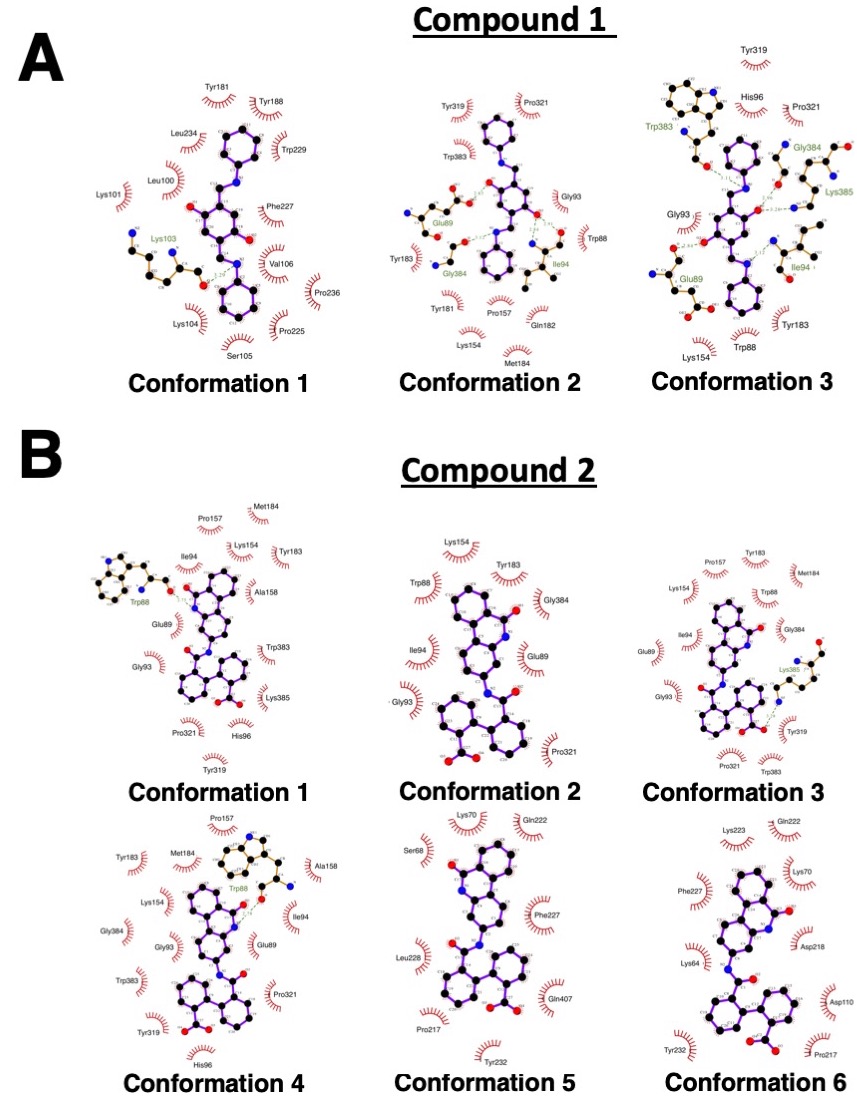
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KEYWORDS: HIV, novel p51 drug target, NNRTIs

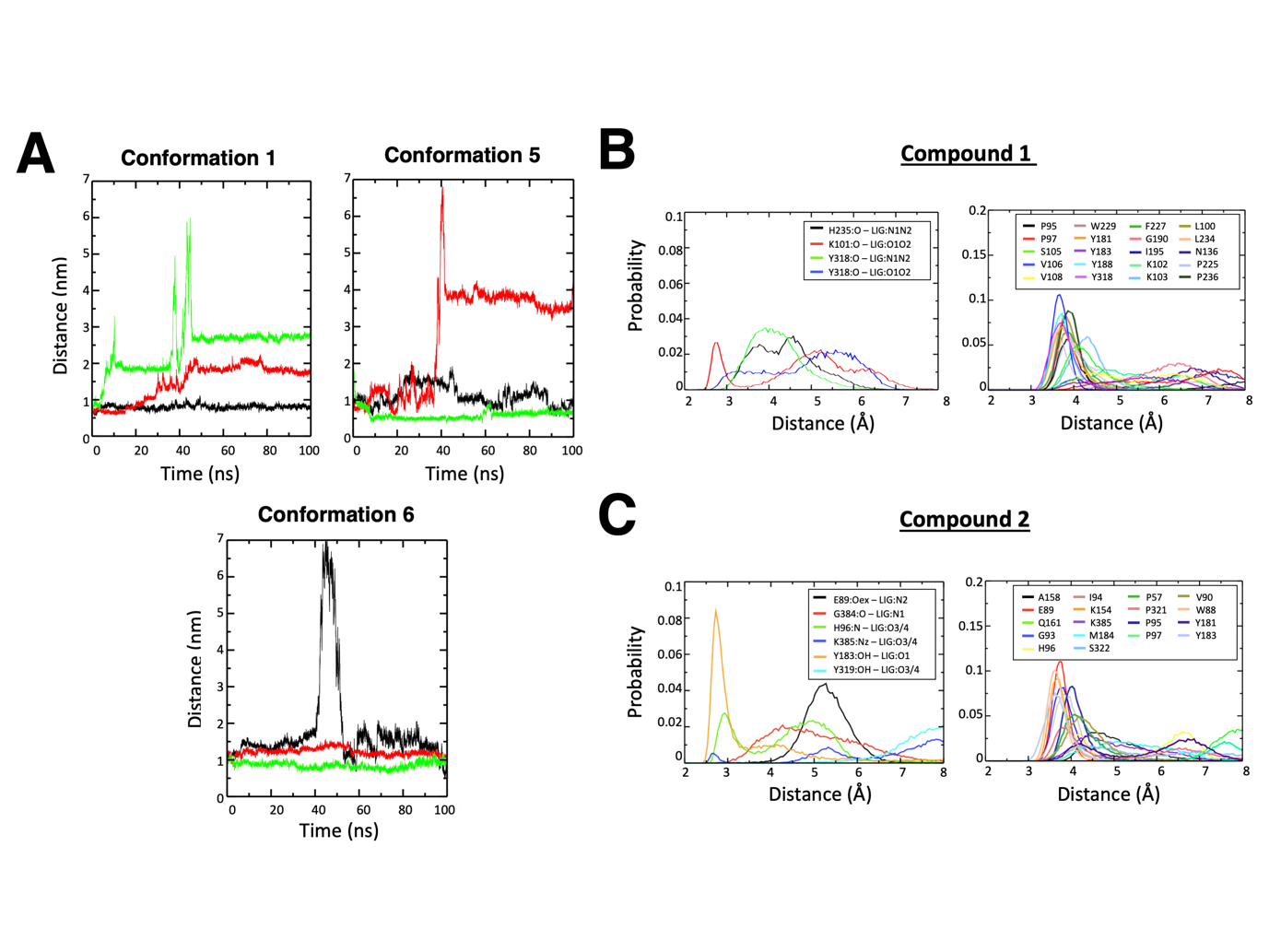
**SUPPLEMENTARY INFORMATION**



**Supplementary Figure S1**. The agarose gel electrophoresis of RT-PCR GAPDH products treated with 40 compounds from the NCI/DTP Diversity Set V. We found two compounds (labeled 8 and 20, thereafter named as compound 1 and 2, respectively) showed the RT inhibition in all independent triplicates. Note that the inhibition by compound 25 was not reproduced in all the independent triplicates and hence it was excluded in our subsequent analysis.



**Supplementary Figure S2**. Initial ligand bound conformations used for different setups of the MD simulations.

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**Supplementary Figure S3.** Molecular simulation analyses of the binding sites for compounds 1 and 2. (A) The center-of-mass distances between the binding sites and compound 2 for three initial conformations that exhibited unstable ligand bound positions, calculated using independent triplicates (black, green and red) of 100 ns trajectories. Probability plots of interactions with their respective binding sites are shown for (B) compound 1 and (C) compound 2.

**Supplementary Table S1**. Decomposition of binding energies for compound 1 in the binding sites on RT p66 and p51 subunits. MM-PBSA calculations were carried out with the program *g\_mmpbsa* [1] for the last 50 ns of each trajectory, using various internal dielectric constants εin for the solute [2-4]. Analyses were performed across independent triplicate simulations, using mean and standard deviation.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound 1 | εint = 2 | | |  | εint = 8 | | |  | εint = 20 | | |
| (kcal/mol) | Conform. 1 (p66) | Conform. 2 (p51)# | Conform. 3 (p51)# |  | Conform. 1 (p66) | Conform. 2 (p51)# | Conform. 3 (p51)# |  | Conform. 1 (p66) | Conform. 2 (p51)# | Conform. 3 (p51)# |
| Total binding energy | -31.5 ± 3.3 | -16.4 ± 2.3 | -14.2 ± 1.7 |  | -39.3 ± 3.9 | -21.7 ± 2.2 | -19.7 ± 2.5 |  | -43.5 ± 4.1 | -27.8 ± 2.0 | -26.0 ± 2.3 |
| van der Waal | -44.2 ± 3.6 | -33.8 ± 1.9 | -33.1 ± 1.3 |  | -44.2 ± 3.6 | -33.8 ± 1.9 | -33.1 ± 1.3 |  | -44.2 ± 3.6 | -33.8 ± 1.9 | -33.1 ± 1.3 |
| Electrostatics | -3.1 ± 0.9 | -3.8 ± 1.0 | -5.6 ± 1.3 |  | -0.8 ± 0.2 | -1.0 ± 0.2 | -1.4 ± 0.3 |  | -0.3 ± 0.1 | -0.4 ± 0.1 | -0.6 ± 0.1 |
| Polar solvation | 20.7 ± 2.0 | 25.2 ± 3.2 | 28.6 ± 1.9 |  | 10.6 ± 0.8 | 17.0 ± 1.8 | 18.9 ± 1.2 |  | 5.9 ± 0.4 | 10.4 ± 1.0 | 11.7 ± 0.9 |
| Non-polar solvation | -4.9 ± 0.4 | -4.0 ± 0.2 | -4.1 ± 0.2 |  | -4.9 ± 0.4 | -4.0 ± 0.2 | -4.1 ± 0.2 |  | -4.9 ± 0.4 | -4.0 ± 0.2 | -4.1 ± 0.2 |

# In both conformations 2 and 3, compound 1 was found to bind to the same site on the RT p51 subunit.

**Supplementary Table S2**. Additional binding analyses for the two compounds in their binding sites on the RT p66 and p51 subunits. Hydrophobic contacts between protein and ligand were calculated using a 4.0 Å cut-off. Hydrogen bonds between protein and ligand were calculated using a cut-off of 3.5 Å and maximum angle between donor – hydrogen atom and acceptor of 30°. The number of water molecules around the ligand was determined using a cut-off of 3.5 Å. Analyses were performed across independent triplicate simulations, using mean and standard deviation.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound 1 | | | |  | Compound 2 | | | |
|  | Number of water molecules | Number of hydrogen bonds | Number of hydrophobic contacts |  |  | Number of water molecules | Number of hydrogen bonds | Number of hydrophobic contacts |
| Conform. 1 (p66) | 2.8 ± 1.5 | 0.6 ±0.6 | 20.4 ± 4.8 |  | Conform. 1 (p51) | 15.2 ± 3.5 | 1.8 ± 1.3 | 13.6 ± 5.1 |
| Conform. 2 (p51) a | 8.2 ± 2.2 | 1.3 ± 0.8 | 12.2 ± 3.2 |  | Conform. 2 (p51) | 13.3 ± 2.6 | 3.0 ± 1.0 | 16.3 ± 3.6 |
| Conform. 3 (p51) a | 7.5 ± 2.2 | 1.3 ± 0.8 | 12.2 ± 3.5 |  | Conform. 3 (p51) | 15.4 ± 2.7 | 1.5 ± 1.1 | 15.0 ± 4.0 |
|  |  |  |  |  | Conform. 4 (p51) | 14.1 ± 2.5 | 2.1 ± 1.3 | 15.7 ± 3.6 |
|  |  |  |  |  | Conform. 5 (p51) b | 15.9 ± 3.6 | 1.7 ± 1.1 | 12.3 ± 5.6 |
|  |  |  |  |  | Conform. 6 (p51) b | 15.2 ± 3.9 | 2.2 ± 1.2 | 12.8 ± 5.7 |

a In both conformations 2 and 3, compound 1 was found to bind to the same site on the RT p51 subunit.

b Secondary binding site (weaker and unsteady) of compound 2 on the p51 subunit.

**Supplementary Table S3**. Decomposition of binding energies for compound 2 in the binding sites on RT p51 subunits. MM-PBSA calculations were carried out with the program *g\_mmpbsa* [1] for the last 50 ns of each trajectory, using various internal dielectric constants εin for the solute. Analyses were performed across independent triplicate simulations. Only results for the three setups that exhibited stable ligand binding (i.e. conformation 2, 3, and 4) are shown. Analyses were performed across independent triplicate simulations, using mean and standard deviation.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound 2 | εint = 2 | | |  | εint = 8 | | |  | εint = 20 | | |
| (kcal/mol) | Conform. 2 (p51) | Conform. 3 (p51) | Conform. 4 (p51) |  | Conform. 2 (p51) | Conform. 3 (p51) | Conform. 4 (p51) |  | Conform. 2 (p51) | Conform. 3 (p51) | Conform. 4 (p51) |
| Total binding energy | -43.7 ± 2.0 | -49.9 ± 3.0 | -45.5 ± 2.2 |  | -13.4 ± 3.6 | -20.1 ± 2.6 | -12.2 ± 5.9 |  | -16.5 ± 2.2 | -17.2 ± 6.3 | -15.1 ± 4.4 |
| van der Waal | -38.7 ± 0.9 | -38.7 ± 0.3 | -38.2 ± 1.1 |  | -38.7 ± 0.9 | -38.7 ± 0.3 | -38.2 ± 1.1 |  | -38.7 ± 0.9 | -38.7 ± 0.3 | -38.2 ± 1.1 |
| Electrostatics | -58.4 ± 8.1 | -57.7 ± 2.8 | -70.3 ± 13.1 |  | -14.0 ± 2.0 | -14.4 ± 0.7 | -17.6 ± 3.3 |  | -5.8 ± 0.8 | -5.8 ± 0.3 | -7.0 ± 1.1 |
| Polar solvation | 57.9 ± 9.9 | 50.9 ± 5.3 | 67.4 ± 13.5 |  | 43.9 ± 5.1 | 37.1 ± 3.6 | 48.0 ± 8.0 |  | 32.5 ± 2.8 | 28.0 ± 2.4 | 34.5 ± 4.6 |
| Non-polar solvation | -4.5 ± 0.1 | -4.1 ± 0.2 | -4.3 ± 0.1 |  | -4.5 ± 0.1 | -4.1 ± 0.2 | -4.3 ± 0.1 |  | -4.5 ± 0.1 | -4.1 ± 0.2 | -4.3 ± 0.1 |

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