# STUDY ON THE INTERACTION MECHANISM OF PENCICLOVIR DRUG ON 3CLpro OF SAR-CoV-2 BY SIMULATION METHODS

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**Abstract**

*Since the outbreak of SAR-CoV-2 infections in Wuhan (China), researched communication is on race to investigate the specific antiviral drug for Covid-19 treatment. 3CLpro main protease is chosen as a protein target because of its high value in preventing the SAR-CoV-2 viral replications. In this study, we hereby aim to clarify the efficiency of Penciclovir in inhibiting the mechanic of 3CLpro target of SAR-CoV-2. Using docking simulation and molecular dynamic simulation (SMD), the interaction of Penciclovir with 3CLpro target was investigated. The results show that Penciclovir strongly interacts with 3CLpro target, and the non-binding interaction plays a more important role than hydrogen bonding in the steady state of the receptor-ligand conformation.*

**Keywords:** *3CLpro, SAR-CoV-2, Penciclovir, docking method, SMD method.*

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# NGHIÊN CỨU CƠ CHẾ TƯƠNG TÁC CỦA THUỐC PENCICLOVIR LÊN 3CLpro CỦA SAR-CoV-2 BẰNG PHƯƠNG PHÁP MÔ PHỎNG

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**Tóm tắt**

*Kể từ khi bùng phát các ca nhiễm SAR-CoV-2 tại Vũ Hán (Trung Quốc), cộng đồng nghiên cứu đang chạy đua để điều tra loại thuốc kháng virus đặc hiệu điều trị Covid-19. 3CLpro Protease main được chọn làm protein thụ thể bởi vì nó có khả năng cao trong việc ngăn chặn sự nhân lên của virus SAR-CoV-2 khi bị ức chế. Trong nghiên cứu này, mục đích chính là làm rõ cơ chế tương tác của Penciclovir với thụ thể 3CLpro của SAR-CoV-2. Sử dụng mô phỏng docking và mô phỏng động lực học phân tử kéo định hướng (SMD), sự tương tác của Penciclovir với thụ thể 3CLpro đã được nghiên cứu. Kết quả cho thấy rằng Penciclovir tương tác mạnh với thụ thể 3CLpro. Kết quả mô phỏng đã thể hiện rõ tương tác không liên kết đóng vai trò quan trọng hơn tương tác liên kết hydro ở trạng thái ổn định của cấu trúc phối tử thụ thể.*

**Từ khóa:** *3CLpro, SAR-CoV-2, Penciclovir, docking method, SMD method.*

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## 1. Introduction

The world has been affected by outbreaks of viral infection such as the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2002-2003, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, Ebola, Zika, and the influenza virus H1N1. The outbreak of SAR-CoV-2 infections from China (Dec-2019) inflected one million cases and killed over 50 thousands of people all over the world. The research by Zhou et al. (2020) showed that the structure of SAR-CoV-2 has been similar to SAR-CoV, shared 96% of the whole genome level of a bat coronavirus, and both viruses belong to the beta genus of coronavirus family (Gorbalenya et al., 2020; Zhou et al., 2020). Because of this closed relation, the novel coronavirus is named SARS-CoV-2. Coronavirus genome contains a glycosylate spike protein (S) which is revealed to play a major function and helps not only the SAR-CoV but also the SAR-CoV-2 virus bind into an angiotensin converting enzyme 2 (ACE2), a protein locates on the host cell's surface membrane. In addition, coronavirus main protease (3CLpro), papain- liked protease (PLpro) and RNA-dependent RNA polymerase (RdRp) are all called non-structural proteins, which are encoded by the SAR-CoV-2 genome. 3CLpro is known to be responsible for the coronavirus replication within the host human cell while the RdRp is related to viral RNS synthesis (Bzówka et al., 2020; Jin et al., 2020).

For the treatment of various herpesvirus infections, Penciclovir is used as a guanosine analogue antiviral drug. It is a nucleoside analogue, which exhibits low toxicity and good selectivity. Penciclovir is inactive in its initial form. Within a virally infected cell, a viral thymidine kinase adds a phosphate group to the Penciclovir molecule; this is the rate-limiting step in the activation of Penciclovir. Penciclovir was found to bind to nsp12 with similar binding energies as that of Remdesivir, which has been used as a therapy for COVID-19 (Dey et al., 2021; Subedi et al., 2021).

Although there is no experimental evidence that Penciclovir binds to 3CLpro, the important role of 3CLpro and initial promising results of Penciclovir in COVID-19 treatment motivates us to investigate the possible binding of Penciclovir to 3CLpro. We therefore performed docking and molecular dynamic simulation (SMD) to calculate the binding efficiency of Penciclovir to 3CLpro. We estimated the binding energy of Penciclovir with 3CLpro is -6.4 kcal.mol$^{-1}$. The non-binding interaction plays a more important role than hydrogen bonding in the steady state of the receptor-ligand conformation. To assess the certainty of the results of the Docking method, SMD method used, the pulling work of Penciclovir from binding site to 3CLpro is 53.2 ± 12.2 kcal.mol$^{-1}$ with $F_{max}$ = 503 ± 54.9 pN.

## 2. Material and methods

### 2.1. The initial structures of Penciclovir and main protease (3CLpro)

Penciclovir is a synthetic acyclic guanine derivative with antiviral activity, mainly used to treat infections from herpes simplex virus (HSV) types 1 and 2. The structure of Penciclovir was taken from PubChem data bank with CID is 135398748 which 2D and 3D conformations are presented in Figure 1. The molecular formula of Penciclovir $C_{10}H_{15}N_5O_3$.

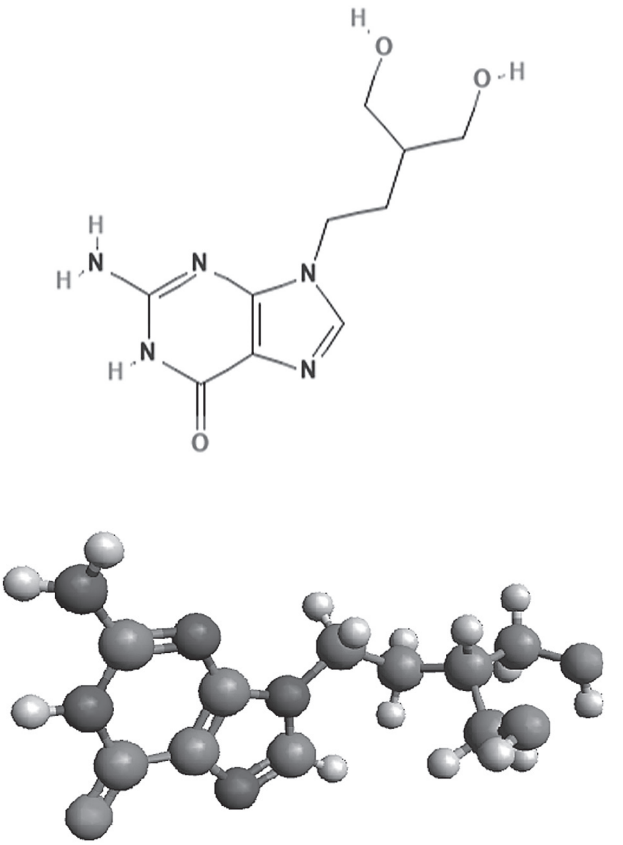

**Figure 1. The 2D and 3D structures of Penciclovir**

The structure of target main protease (3CLpro) was obtained from protein data bank (PDB) with PDB ID 6LU7, It showed in Figure 2.

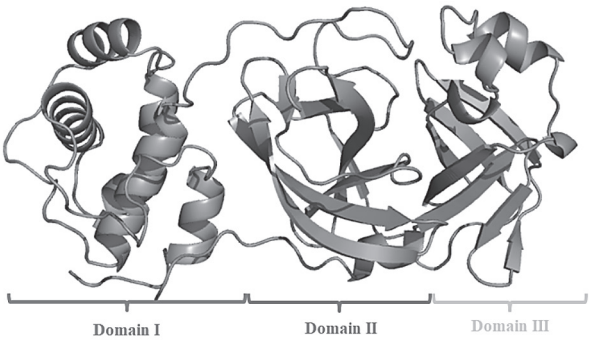


**Figure 2: The initial structure of target 3CLpro (6LU7)**

## 2.2. Methods

*2.2.1. Docking method*

PDBQT files prepared by AutoDock Tool 1.5.4 (Sanner, 1999) were used to dock Penciclovir to target 3CLpro (6LU7) (Liu et al., 2020). The Autodock Vina version 1.1 (Trott & Olson, 2010) was utilized for docking simulation. For global search, the exhaustiveness was set to 600 which is high enough to achieve reliable results. The dynamics of receptor atoms were neglected. Twenty binding modes have been generated starting from random configurations of ligand that had fully flexible torsion degrees of freedom. The box was chosen big enough to cover the entire receptor with minimal distance between ligand and target of 1.4 nm.

*2.2.2 Steered molecular dynamics*

The steered molecular dynamics (SMD) method was developed to study mechanical unfolding of biomolecules (Israelewitz et al., 2001, Kumar & Li, 2010) and ligand unbinding from receptor along a given direction (Grubmüller et al., 1996). Because the predictive power of the docking method is limited the SMD method was employed to refine docking results as a next step in the multi-step screening procedure. Overall, a spring with spring constant k is attached to a dummy atom at one end and to the first heavy atom of ligand in the pulling direction at another end. Moving along the pulling direction with a constant loading speed v the dummy atom experiences elastic force $F = k(\Delta z - vt)$, where $\Delta z$ is the displacement of pulled atom from the starting position. The initial structure for SMD simulation was used equilibrated configurations. To preserve the overall structure of targets, the C-alpha atoms are restrained using harmonic potential with spring constant k = 1000kJ. $nm^{-1}.mol^{-1}$. In this work, the pulling rate is 0.015 $nm.ps^{-1}$ and the pulling constant is 600kJ/nm/mol. The pulling force put on the center of mass of Penciclovir with the direction along the z axis.

*2.2.3. The pulling direction*

Using MSH method (Vuong et al., 2015), chose the easiest path for ligand to exit from receptor as the pulling direction. It is shown in Figure 3. After equilibration, to completely pull the ligand out of the binding site, 500 ps SMD runs were carried out in NPT ensemble. To obtain reliable results, five independent trajectories were performed with different random seeds. In the SMD method the maximum force $F_{max}$ in the force-extension/time profile was chosen as a score for binding affinity, the larger is $F_{max}$, the stronger is the ligand binding.

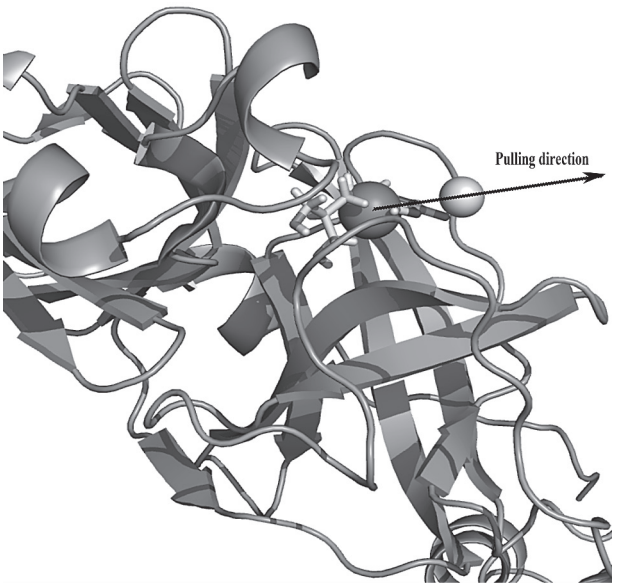


**Figure 3. The pulling direction of Penciclovir was obtained by MSH**

## 3. Results and discussion

### 3.1. Docking results

Figure 2 showed the cartoon representation of 3CLpro target. Because binding site of Penciclovir in 3CLpro target was identified a mechanism-based inhibitor N-[(5-methylisoxazol-3-yl) carbonyl] alanyl-l-valyl-n~1~-((1r,2z)-4-(benzyloxy)-4-oxo-

1-{[(3r)-2-oxopyrrolidin-3-yl] methyl}but-2-enyl)-l-leucinamide (N3) by computer-aided drug design, and then determined the crystal structure of Main pro of SARS-CoV-2 in complex with this compound, a sufficiently large grid box is created around the detected binding pocket with the spacing 0.375 Å. The box size is 20 x 20 x 20 (Å). A big grid box allows the ligand to traverse a larger conformational space on the protein surface and perform the blind docking process. In Figure 4, the configurations obtained from the best docking modes for Penciclovir in complexes with 3CLpro (6LU7). With the best docking mode, the docking binding energy of Penciclovir with 3CLpro is -6.4 kcal.mol$^{-1}$ in Table 1, implying that Penciclovir pretty strong binds into 3CLpro. The *in vitro* experiment showed that the $EC_{50}$ value of Penciclovir for SARS-CoV-2 is 95.06 μM. Using the formula $\Delta G^{exp} = RTln(EC_{50})$, where gas constant R = 1.987 x 10$^{-3}$ kcal.mol$^{-1}$, T = 300 K, and $EC_{50}$ is measured in M, the result was obtained $\Delta G^{exp}$ = -5.5 kcal.mol$^{-1}$, which is roughly consistent with our docking result.

From Figure 4, it showed that Penciclovir binds into hole of binding site of 3CLpro in the space between domain II and III. Penciclovir was painted by cyan and blue while N3-ligand of 3CLpro colored pink. Figure 4 also presented that Penciclovir is located more deeply than N3. Those may be part of the reason for why Penciclovir good binding into 3CLpro.

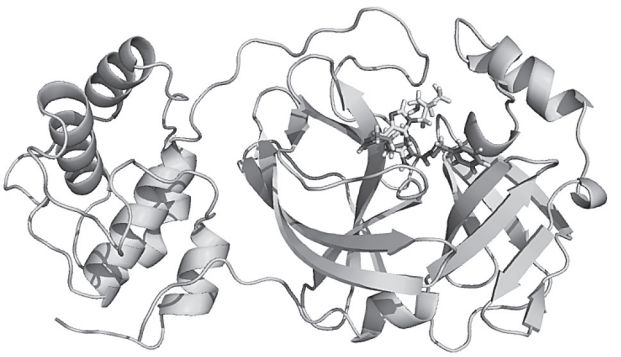

**Figure 4. The binding sites of Penciclovir in complexes with main protease for the best mode of docking simulation**

**Table 1. The 09 configurations of Penciclovir bind into hole of binding site of 3CLpro with difference oot-mean-square deviation (RMSD)**

| Penciclovir | $\Delta E_{bind}$ (kcal.mol$^{-1}$) | Penciclovir | $\Delta E_{bind}$ (kcal.mol$^{-1}$) | Penciclovir | $\Delta E_{bind}$ (kcal.mol$^{-1}$) |
|---|---|---|---|---|---|
| Mode_1 | -7.3 | Mode_4 | -6.3 | Mode_7 | -6.4 |
| Mode_2 | -7.0 | Mode_5 | -5.8 | Mode_8 | -5.2 |
| Mode_3 | -6.5 | Mode_6 | -6.5 | Mode_9 | -6.2 |

In the complex of Penciclovir with 3CLpro, the results showed in Figure 5. Penciclovir forms 07 hydrogen bonds (HB) and 25 non-bond contacts (NBs). The residues of 3CLpro which form HBs with Penciclovir are Ser144, Leu141 (connect with N3, N5 atoms of Penciclovir), Tyr54, Asp187, His164, Cys145 and non-bonded contacts at residues Glu166, Met49, His41, Met165, Gln189, Gly143, and Asn142. While the residue Leu141 is the shortest with Penciclovir ligand. These results indicate that non-bonded contacts dominate the interaction between Penciclovir and main protease (3CLpro).

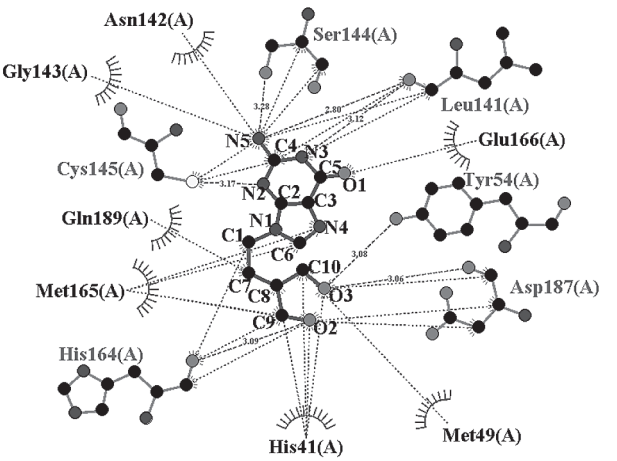


**Figure 5. The hydrogen bonds are in green, non-bonded contacts are in red lines**

### 3.2. SMD results

Using the MSH (Mai and Li, 2011, Vuong et al., 2015) one can obtain several possible pulling directions but the easiest pathway with the lowest rupture force $F_{max}$ was chosen. SMD runs were performed for ten independent trajectories and the results were averaged over all trajectories. Typical force-time curves are presented in Figure 6 showing the sensibility of rupture force on SMD runs. The SMD results are shown in Table 2.

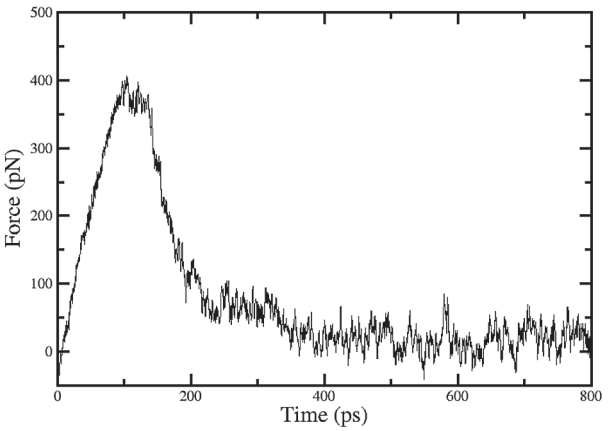


**Figure 6. Force-time profiles of a representative SMD trajectory of 3CLpro**

**Table 2. The ranking of rupture force ($F_{max}$) was obtained by SMD method.**

| No. | Work (kcal.mol$^{-1}$) | $F_{max}$ (pN) | $F_{max}$ time (ps) |
|---|---|---|---|
| 1 | 105.5 | 488.0 | 105.5 |
| 2 | 134.4 | 618.9 | 134.4 |
| 3 | 105.2 | 500.8 | 105.2 |
| 4 | 93.7 | 432.2 | 93.7 |
| 5 | 103.5 | 522.5 | 103.5 |
| 6 | 120.4 | 529.6 | 120.5 |
| 7 | 167.8 | 473.2 | 167.8 |
| 8 | 123.4 | 567.5 | 123.4 |
| 9 | 97.5 | 461.6 | 97.5 |
| 10 | 93.1 | 442.8 | 93.1 |
| Average | 53.2 ± 12.2 | 503 ± 54.9 | 114.5 ± 21.9 |

Because the SMD method is shown to be more accurate than the docking approach. By SMD method, the profiles of pulling force with time and displacement are shown in the Figure 6. The pulling none-equilibrium work of Penciclovir from binding to main protease (3CLpro) is 53.2 ± 12.2 kcal.mol$^{-1}$ with $F_{max}$ = 503 ± 54.9 pN at $F_{max}$ time 114.5 (ps). These values are close to CID 131801415 compound (Thai et al., 2022).

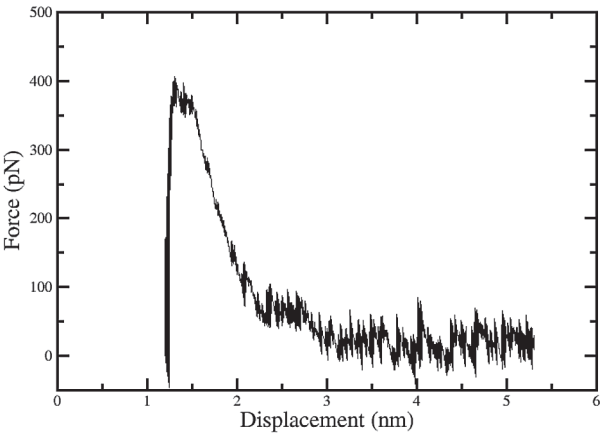


**Figure 7. The force is plotted as a function of ligand displacement**

In this study we did not detail the results of SMD; they will be presented in follow-up research.

### 4. Conclusions

Using docking simulations, we obtained the binding energies of Penciclovir and 3CLpro. The results showed that Penciclovir strongly interacts with 3CLpro target of SAR-CoV-2, and the non-binding interaction plays a more important role than hydrogen bonding in the steady state of the receptor-ligand conformation. Besides, combining docking and SMD method, the SMD is shown to be more accurate than the docking approach, which showed rupture force. The results of SMD method presented that pulling none-equilibrium work of Penciclovir from binding to main protease (3CLpro) is productive. That means Penciclovir is a potential drug for the treatment of Covid-19. Therefore, we recommend it to in vitro and in vivo studies. The reliability of SMD approach has been also checked by computation of binding free energies for seven systems using the MM-PBSA method, which has yet to present in this paper./.

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