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Research Article

# The Inhibitory Activity of Ruxolitinib Against COVID-19 Major Protease Enzyme and SARS Cov-2 Spike Glycoprotein: A Molecular Docking Study

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**Abstract -** Ruxolitinib ($C_{17}H_{18}N_6$) is a Janus kinase (JAK) inhibitor that inhibits JAK1, JAK2, and JAK3 and with its tyrosine kinase inhibitor function It is the first drug approved for use in the treatment of myelofibrosis. The possible conformations of the ruxolitinib molecule were searched using PM3 technique and the Spartan06 software. The estimated molecular energies of the Ruxolitinib conformers, obtained by the variations in dihedral angles, were compared, and the most stable conformer was determined. To enlighten the inhibitory activity of Ruxolitinib agaist the apo (PDB ID: 6M03) and holo (PDB ID: 6LU7) forms of the main protease enzyme ($M^{pro}$) of COVID-19 and the SARSCoV-2 spike glycoprotein (PDB ID: 6VXX), molecular docking simulations were performed. The binding affinities and binding modes were determined. The binding free energies of ruxolitinib and 6M03, 6LU7, 6VXX targets calculated by the combination of Molecular Mechanics/Generalized Born Surface Area (MMGBSA) and Molecular Mechanics/Poisson-Boltzmann Surface Area (MM-PBSA) methods {MM/PB(GB)SA approach}, were found to be -22.24, -19.96 and -22.44 kcal/mol, respectively.

**Keywords:** Ruxolitinib, SARSCoV-2, tyrosine kinase, molecular docking

## 1. Introduction

Human coronaviruses, known as CoV, are enveloped positive-stranded RNA viruses belonging to the Nidovirales family. It is mostly responsible for upper respiratory and digestive tract infections [1]. SARS-CoV-2 is made up of four primary structural proteins: spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) glycoprotein, as well as various auxiliary proteins. The spike, also known as S glycoprotein, is a transmembrane protein located in the virus's outer surface. There are several variants of the coronavirus, and like the other variant, the SARS-CoV-2 genome encodes spike (S) glycoproteins that protrude from the surface of mature virions. These spike glycoproteins are actively involved in the fusion of the virus, its entry into the host cell and its attachment. Its surface location is the reason why spike glycoproteins are the main target for neutralizing antibodies and host immune responses [2]. Apart from these properties, the Spike protein is also the first target for vaccine design, as we can show its central roles in viral infection and eliciting protective humoral and cell-mediated immune responses in hosts during infection [2-4]. Spike protein helps envelope viruses bind to host cells in the lower respiratory tract by forming homotrimers that protrude from the viral surface. It also shares common structural, topological, and mechanical properties with other class I fusion proteins, including the SARS-CoV-2 Spike glycoprotein, HIV envelope (Env) glycoprotein, and influenza virus hemagglutinin (HA) [5, 6].

The Ruxolitinib molecule ($C_{17}H_{18}N_6$) is one of the few hemato-oncology drugs that works by affecting epigenetic mechanisms. Ruxolitinib, which was marketed under the name Jakafi in 2011, is the first chemical agent approved for the treatment of myelofibrosis [7]. Ruxolitinib shows its

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effectiveness by preventing the function of molecules called JAK1 and JAK2 (janus kinase), which are effective in blood cell production and immune response, that is, it has immunosuppressive properties. Ruxolitinib (INCB018424) is a potent, optional JAK1 and JAK2 inhibitor. Its main effect is inhibition of cell division and induction of apoptosis by preventing JAK from being able to phosphorylate STAT [8].

Cytoplasmic Janus protein tyrosine kinases (JAKs) are important components of various signal transduction pathways that govern cellular survival, proliferation, differentiation and apoptosis. The primary functional feature of JAK kinases is phosphorylation of cytokine and growth factor receptors. Over activation of JAK kinases has been associated with tumorigenesis. As opposed to, loss of JAK kinase function, on the other hand, has been linked to diseases such as severe combined immunodeficiency [9].

Patients with severe COVID-19 experience elevated plasma levels of proinflammatory cytokines that can cause a cytokine storm, followed by extensive immune cell infiltration into the lungs, causing alveolar damage, decreased lung function, and rapid progression to death. In addition, ruxolitinib, a powerful and optional JAK1 and JAK2 inhibitor, has started to take its place as a current issue in the treatment of diseases associated with chemokines and cytokines. As most of the upregulated cytokines signal via Janus kinase (JAK)1/JAK2, inhibition of these pathways by ruxolitinib has the potential to attenuate the COVID-19-associated cytokine storm and reduce mortality [9,10].

Yeleswaram et al. [11] investigated the effect of Ruxolitinib on the inhibition of cytokine signaling. In this study, Ruxolitinib has been shown to reduce cytokine levels and improve outcomes and reported that the patients who were given Ruxolitinib showed a numerically faster recovery from COVID-19 [11].

$M^{pro}$ major protease inhibits the cleavage of viral polyproteins and inhibits the spread of infection. Therefore, it is a potential protein target [12-14].

To explore the usage of the Ruxolitinib in the treatment of COVID-19 and to enlighten its interaction mechanism with apo and holo forms of SARSCoV-2 main protease enzyme ($M^{pro}$) and with SARSCoV-2 spike glycoprotein, molecular docking simulations were performed. The binding affinities and binding modes were determined.

## 2. Materials and Calculations

Molecular modeling was carried out using the Spartan06 software [15] and the PM3 method [16-19]. The CAVER software [20] was used to identify potential binding sites on the receptor's surface. On the identified active sites, Molecular Docking investigations were done by using AutoDock-Vina software [21].

## 3. Results and Discussions

### 3.1. Structure

**Table 1** shows the relative energies of the obtained three possible conformers of Ruxolitinib by conformational analysis and **Figure 1** shows the molecular models these conformers.

**Table 1.** The relative energies of three lowest energy conformers of Ruxolitinib, obtained by conformational analysis.

| Conformers | Relative energy (kj/mol) |
|---|---|
| Conformer I | 0 |
| Conformer II | 0.44 |
| Conformer III | 2.48 |


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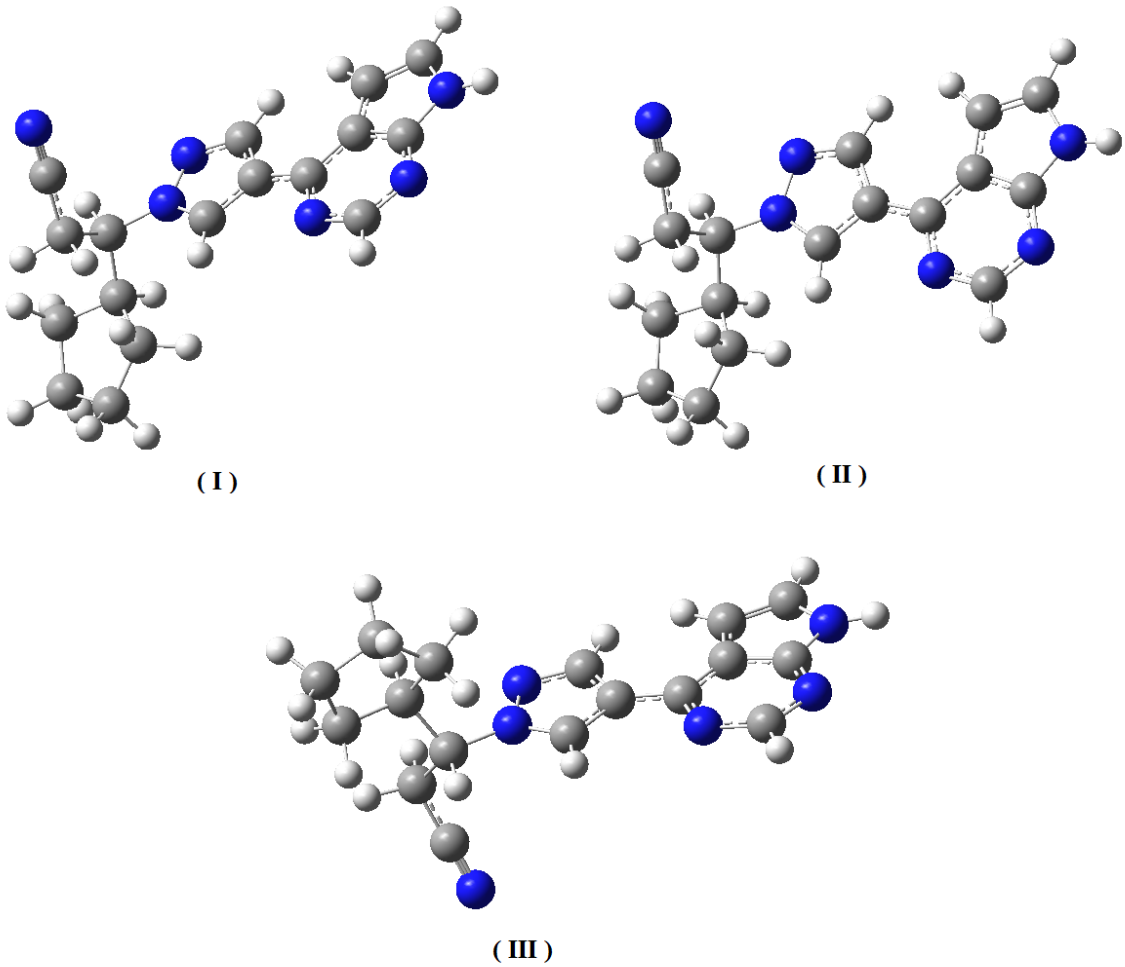


**Figure 1.** The three lowest energy conformers of the Ruxolitinib, obtained by conformational analysis.

### 3.2. Molecular Docking

To demonstrate the antiproliferative effects of ruxolitinib in our current study, molecular docking simulations were performed with the most stable structure of Ruxolitinib obtained, with SARSCoV-2 main protease enzyme (Mpro) apo/holo forms and then with SARSCoV-2 spike glycoprotein, and binding affinities and binding modes were determined.

The spike glycoprotein crystal structure (PDB ID: 6VXX), the apo form of Mpro (PDB ID: 6M03), and the holo form of Mpro (PDB ID: 6LU7) were all retrieved from the protein database [22-24], and AutoDockVina was used to perform docking tests with Ruxolitinib. Ruxolitinib's most active site was identified, and docking simulations with the apo/holo forms of the SARSCoV-2 major protease enzyme (Mpro) and the SARSCoV-2 spike glycoprotein revealed and depicted in three dimensions in Figure 2. And Ruxolitinib interactions with three receptors are shown in Figures 2-4.

The interactions between the apo form of Mpro and Ruxolitinib (shown in Figure 2) are as follows:

3.46 Å length carbon hydrogen bond between ruxolitinib and His41; 2.31 Å long hydrogen bond with Cys44; 5.12 Å long pi-alkyl interaction with Met49.

And also, 2.42 Å long hydrogen bond with Gly143, as well as 5.2 Å long pi-alkyl interaction with Cys145 and 2.21 Å long hydrogen bond with Glu166 are formed. -7.5 kcal/mol was determined as the binding affinity.


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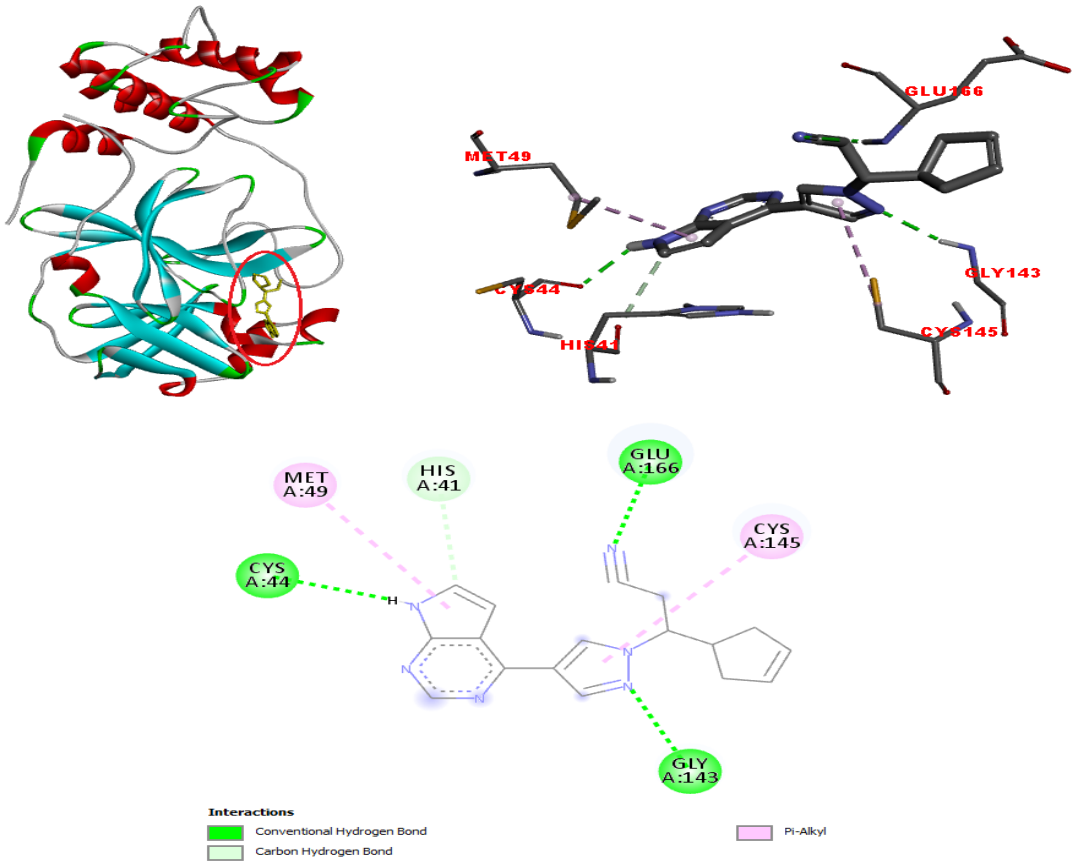


**Figure 2.** The 3D docked representations of the most stable conformer of Ruxolitinib in the active site of **apo** form of main protease enzyme ($M^{pro}$) ( ΔG = -7.5 kcal/mol).

Sagaama et al. [25] performed molecular docking simulation of Succinic acid (SA) with 6m03 and found that SA interacted with Ser144, Cys145, and Glu166 through hydrogen bonding interactions. This result is consistent with our findings.

**Figure 3** depicts the interactions between Ruxolitinib and the active site of the **holo** version of $M^{pro}$ in 3D docking representations. The binding affinity of Ruxolitinib docked with the holo form of SARSCoV-2 main protease enzyme ($M^{pro}$) is found to be -7.2 kcal/mol.

Between the ruxolitinib molecule and Val104, 5.17 Å long pi-alkyl, with Ile106, 5.37 Å long pi-alkyl; 2.64 Å long pi-donor hydrogen bond with Gln110; It was found also that with Thr292, 2.05 Å long hydrogen bond and with Phe294, 4.09 Å long pi-alkyl interaction.


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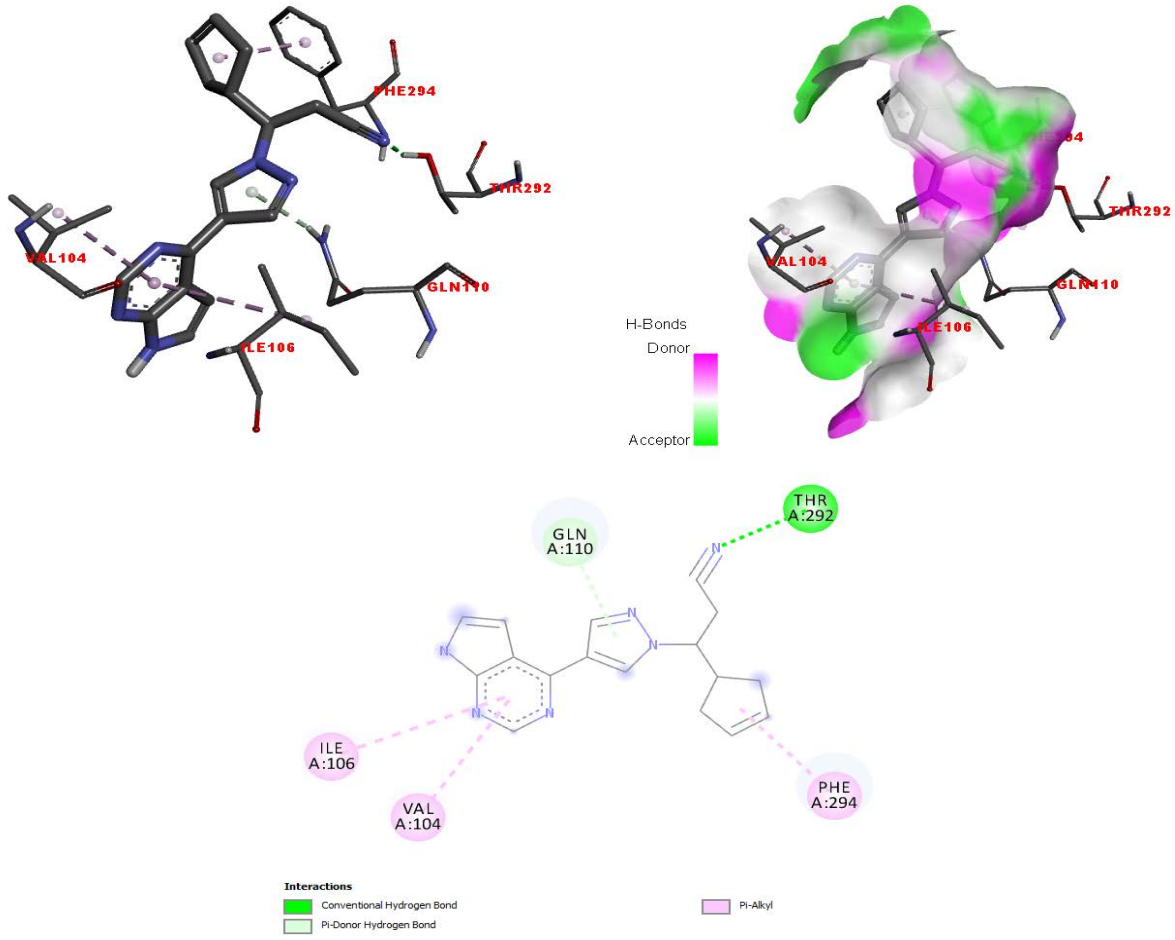


**Figure 3.** The most stable conformer of Ruxolitinib in the active site of **holo** form of COVID-19's main protease enzyme (Mpro) ( -7.2 kcal/mol) in 3D docked representations.

Beura and Chetti [26] investigated the interactions of Chloroquine and its derivatives with SARS-CoV-2, by molecular docking simulations. In this study, it was reported that Chloroquine derivatives (CQD15 and CQD16) formed hydrogen bonds with Gln110, Thr-111, and Asp-153 as well as a pi-pi interaction with Phe8 [26]. The results are in accord with our findings.

The docking simulations of Ruxolitinib with SARSCoV-2 spike glycoprotein revealed the binding affinity as -7.5 kcal/mol. As seen in **Figure 4**, the interactions of ruxolitinib molecule with SARSCoV-2 spike glycoprotein are as follows:

5.2 Å long pi-alkyl interaction with Trp104; alkyl interaction with Ile119 (4.12 Å); hydrogen bonding interaction with Asn121 (2.68 Å); Pi-alkyl interactions (4.55 and 4.81 Å) and pi-sigma interaction (3.92 Å) with Val126; pi-donor hydrogen bond interaction with Ser172 (2.98 Å); Hydrogen bonding with Arg190 (2.57 Å); and alkyl interaction with Ile203 (4.71 Å).

Veerasamy et al. [27] investigated the potential of andrographolide derivatives against COVID-19, and molecular docking simulation of the andrographolide derivative compound 15 into 6vxx target revealed hydrophobic interactions with Val126, Leu226 and Val227 and hydrogen bonds with Asn121 and Arg190 [27].


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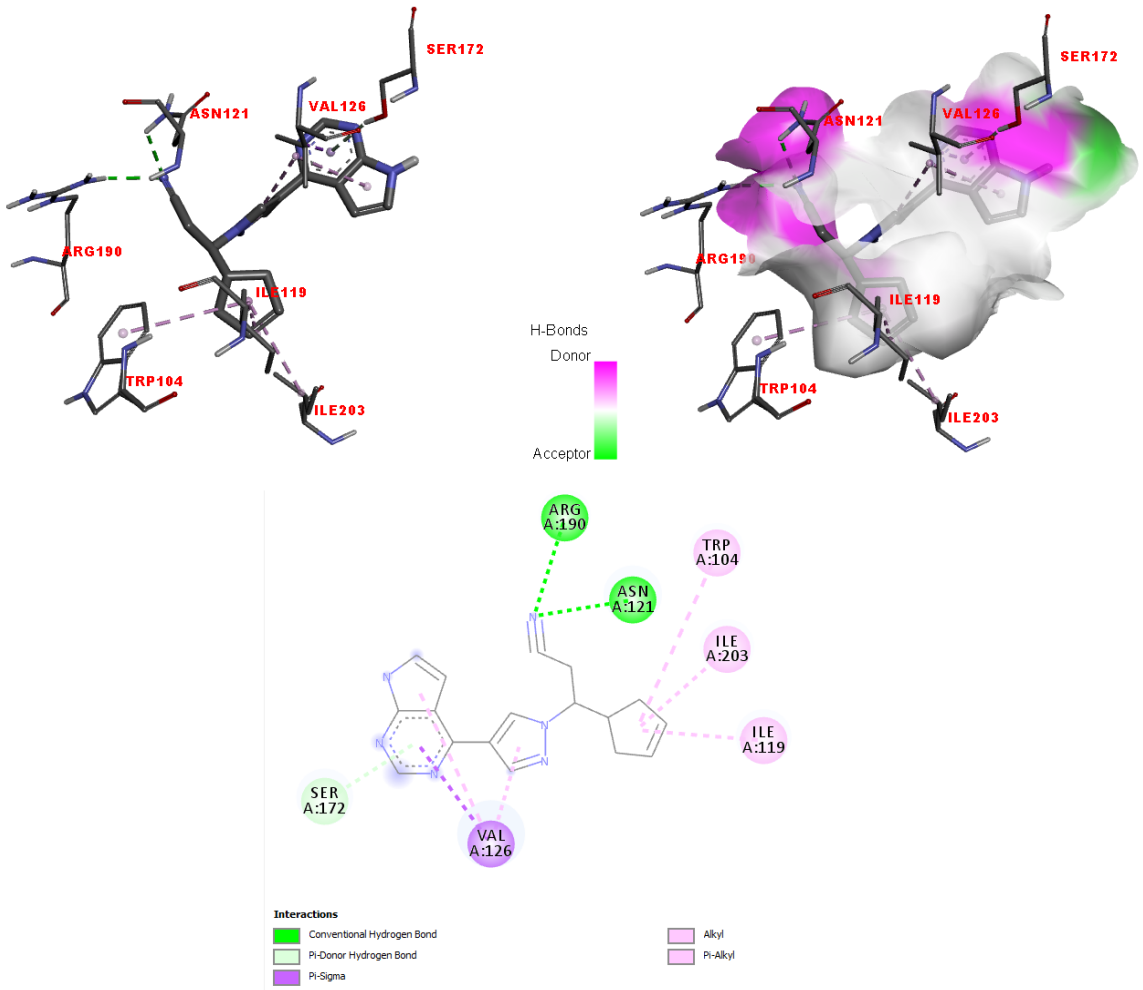


**Figure 4.** The most stable conformer of Ruxolitinib in the active site of the SARSCoV-2 spike glycoprotein ( -7.5 kcal/mol) in 3D docked views.

In this study binding free energy of the most stable ligand protein complexes, that were identified by molecular docking studies, were also calculated. The MM/PBSA and MM/GBSA (molecular mechanical energies combined with Poisson-Boltzmann or generalized Born and surface area continuous solutions) methods estimate the free energy of binding small ligands to biological macromolecules [28-33]. By utilizing the MM/PB(GB)SA methods with the GAFF2 and ff14SB force field combination and the GB6 process, the predicted binding free energies of the ruxolitinib-Apo form, ruxolitinib-Holo form, and ruxolitinib-spike glycoprotein were -22.24, -19.96, and -22.44 kcal/mol, respectively.

## 4. Conclusions

Molecular docking has become an increasingly important tool for drug discovery. All docking approaches have the same goal: to anticipate the structure of the resultant complex and the biological activity of a given ligand. In this study, we present a brief introduction to evaluate Ruxolitinib's inhibitory action, the first step was to do a conformational analysis with PM3 to find the most stable conformer, and followed by docking simulations with both the apo/holo forms of $M^{pro}$ and spike glycoprotein. One goal of this work is to estimate the absolute binding affinity for protein-ligand complexes and the accuracy of identifying the correct binding positions generated from molecular docking programs. In this case, Ruxolitinib was shown to have the most stable structures, with binding affinities of -7.5, -7.2, and -7.5 kcal/mol with the apo/holo forms of the main protease enzyme (Mpro) and spike glycoprotein, respectively. In pharmaceutical chemistry, the study of binding


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affinities is of great interest, and the discovery of a novel drug often involves the synthesis and testing of hundreds of drug candidates. Naturally, it would be beneficial if computational approaches could determine binding affinities quickly and reliably.

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