Research Article

In Silico Screening, In Vitro M\textsuperscript{PRO} Inhibitory, and Adjunctive Therapy Value of Minocycline for the Treatment of COVID-19

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What Is Known and Objective. Novel coronavirus disease (COVID-19) is still ravaging globally since its first discovery in 2019. With the continuous emergence of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) mutant strains, therapeutic entities are still needed to be discovered. This study was to explore SARS-CoV-2 inhibitors and therapeutic entities for COVID-19.

Methods. Based on Lipinski’s rule of 5, a small-scale “old” drug database (clinical drugs being used in Ordos Central Hospital) was established, and in silico screening of M\textsuperscript{PRO} inhibitors was conducted. Binding affinity and interaction as well as structure-affinity relationship were analyzed. Furthermore, molecular dynamic (MD) simulation of the selected drugs was performed to understand the conformational changes in docked complex. In vitro M\textsuperscript{PRO} inhibition tests were performed according to established methods. Finally, literature review of potential “old” drug for the treatment of COVID-19 was conducted. Results and Discussion. The antibiotic minocycline, an inhibitor of bacterial ribosomal RNA, was screened out which showed the highest binding affinity (\(-9.6\text{kcal/mol}\)). Beside the hydrogen bond with Cys145 and hydrophobic interactions, minocycline formed a pi-cation with His41, which strongly supported minocycline as a Michael addition acceptor to bind with the catalytic site of M\textsuperscript{PRO}. MD simulation results show that minocycline displayed less conformational changes. The structure-affinity relationship was summarized based on minocycline analogs, and minocycline showed in vitro M\textsuperscript{PRO} inhibitory activity with \(\text{IC}_{50}\) of 5 mM. More importantly, the literature review found that minocycline had both in vitro and in vivo broad-spectrum antiviral as well as anti-inflammatory activities, and the levels of a broad spectrum of biological markers during minocycline administration were opposed to those of COVID-19 conditions (both severe and nonsevere). What is New and Conclusion. Minocycline deserves an adjunctive therapeutic option for COVID-19 condition (both severe and nonsevere). This study shed a new light on drug-repurposing strategy for the viral disease.

1. What Is Known and Objective

Until now, globally ongoing epidemic of the novel coronavirus (severe acute respiratory syndrome-coronavirus-2, SARS-CoV-2) disease (COVID-19) has infected more than 765,000,000 people and killed more than 6,900,000 patients according to the World Health Organization (WHO). Common symptoms of COVID-19 include fever, cough, shortness of breath, and dyspnea. In more severe cases, infection causes pneumonia, severe acute respiratory syndrome, kidney failure, or even death [1]. Although the vaccinated population in the world has exceeded 13,344,000,000 to end this catastrophic epidemic, more treatments other than vaccines need to be developed [2]. Remdesivir is the only drug approved by the Food and Drug Administration (FDA) for the treatment of COVID-19. Ritonavir-enhanced nirmatrelvir (Paxlovid), molnupiravir, and certain anti-SARS-CoV-2 monoclonal antibodies (MABs) have received emergency use authorizations from the FDA for the treatment of COVID-19. Antiviral therapies...
are being investigated for the treatment of COVID-19, and the drugs prevent viral replication through various mechanisms, including blocking SARS-CoV-2 3-chymotrypsin-like protease (3CLpro, also named as Mpro) and RNA-dependent RNA polymerase (RdRp), causing viral mutations. Besides, the previous study found that the combination of brequinar (BRQ), a dihydroorotate dehydrogenase (DHODH) inhibitor, and dipyridamole (DPY), a remedial pathway inhibitor, expresses a strong synergistic antiviral activity to treat SARS-CoV-2 [3]. As SARS-CoV-2 mutant strains continue to emerge, new therapeutic entities are still needed to be discovered.

Nonstructural proteins (NSPs) are involved in SARS-CoV-2 RNA transcription and translation, protein synthesis, protein processing and modification, virus replication, and host infection [4]. In NSPs, Mpro is automatically cleaved from polyproteins to produce mature enzymes and further cleaves downstream NSPs at 11 sites to release NSP4–16 from polyproteins to produce mature enzymes and further cleaves downstream NSPs at 11 sites to release NSP4–16 [5]. The structure and catalytic mechanism of SARS-CoV Mpro allows it as a promising target for anti-coronavirus drug development. To develop an anti-SARS-CoV-2 drug from scratch is theoretically time consuming [6]. We can test existing broad-spectrum antivirals for their metabolism, used dosages, efficacy, and side effects. However, the side effects of broad-spectrum antivirals should not be underestimated [7]. Because “old drugs” have been prepared, the medication has sufficient experience, and the safety and pharmacokinetic parameters are well known, screening for SARS-CoV-2 therapeutic candidates from existing clinical “old drugs” is always a good strategy [8, 9]. From this strategy, Masitinib was recently discovered as a broad coronavirus 3CL inhibitor that blocked SARS-CoV-2 virus replication [10].

In this study, we established a small-scale “old drug” database (clinical drugs being used in Ordos Central Hospital) according to Lipinski’s rule of 5 and conducted in silico screening of Mpro inhibitors by molecular docking. Binding affinity and interaction as well as structure-affinity relationship were analyzed to better understand the potentiality. The in vitro Mpro inhibition test was conducted based on established methods. Finally, literature support for the potentiality of anti-SARS-CoV-2 and treatment of COVID-19 was reviewed and analyzed. This study will provide contribution to the transient ongoing infectious diseases.

2. Methods

2.1. Pharmacophore Study of the Co-Crystal Ligand N3. The crystal structure of SARS-CoV-2 Mpro in complex with designed ligand N3 (2.1 Å resolution, PDB code: 6LU7) was determined by Professors Zihe Rao and Haitao Yang’s research team from Shanghai Tech University [11]. The protein coordinates of the Mpro used in this study were donated by Zihe Rao et al. in Jan 28th, 2020. Based on the structure, key helices/loops, amino acid residues, and hydrophobic interactions in the binding site were investigated. The pharmacophore of N3 was summarized and was used as a control in the following in silico study.

2.2. Drug Database Establishment. According to Lipinski’s RULE of 5, we established a small-scale database including 135 drugs clinically being used in Ordos Central Hospital [12]. Requirement-reached drug 2D structures were drawn by ChemDraw Professional 17.0 software (CambridgeSoft Corporation, Cambridge, MA, USA). The 2D structures of candidates were converted into 3D structural data by Chem3D ultra 17.0 software (CambridgeSoft Corporation, Cambridge, MA, USA), and all structures of the ligands were energy-minimized.

2.3. Molecular Docking by AutoDock Vina. We applied a workflow for molecular docking which was described in our previous work [13–15]. The chain B (co-crystal ligand N3 in 6LU7) and chain C (water molecules) were deleted, and chain A was prepared for docking within the molecular modeling software package Chimera 1.10.2 (National Institutes of Health, Bethesda, MD, USA) [16]. Adding polar hydrogens and Kollman charges, Gasteiger computing and grid box parameters defining were done using MGL tools 1.5.6 (The Scripps Research Institute, La Jolla, CA, USA) [17, 18].

All the ligands were set as flexible, and the receptor was set as rigid. Docking calculations were performed using AutoDock Vina 1.1.2 software (The Scripps Research Institute, La Jolla, CA, USA) [19]. A search grid box was set to cover the whole surface of Mpro protein to collect all possible orientations and conformations of the ligand paired with the protein (including compounds outside the active site). For which, the center was set as center_\(x = -23.982,\) center_\(y = 12.114,\) center_\(z = 57.466,\) and the size was set as size_\(x = 58,\) size_\(y = 78,\) size_\(z = 66.\) Spacing angstrom was set as 1,000, and the exhaustiveness was set as 100. The default settings and the AutoDock Vina scoring function were applied.

Totally, 9 binding modes were generated by AutoDock Vina for each compound, and the mode (even outside the active site) with the highest binding affinity was selected as the most predictable. Visual investigation and analysis of ligand-protein interactions were performed using PyMOL V.1.5 (Schrodinger LLC, New York, NY, USA).

2.4. Re-Docking by Discovery Studio. The 3D protein structure of Mpro was defined as the receptor and optimized by hydrogenation, dehydration, and removing redundant residues. Location of N3 in the cocystal was defined as the active site with a radius as 13.890841 covering the binding region. The X, Y, and Z centers were set as \(-10.797, 12.536,\) and 68.905, respectively. Molecular structures of ligands were prepared and converted to 3D structures. The molecular docking was performed using CDOCKER tool. -CDOCKER_ENERGY and -CDOCKER_INTERACTION_ENERGY were used to score the interaction between the receptor and ligand. Discovery Studio (DS) 2022 software (BIOVIA, San Diego, CA, USA) was used for the docking, visualization, and analysis [20].
2.5. Molecular Dynamics (MD) Simulation. MD approach is widely used to assess atoms’ behavior, structural stability, and study the conformational changes on atomic level. Herein, after the molecular docking, MD simulation was performed on the compounds with best affinity by DS. The complexes were minimized using the CHARMM force field. During the solvation process, default waterbox size options were selected, and the waterbox size was adjusted to match the protein’s size using a rectangular waterbox type with an edge distance of 7.0 Å. To neutralize the system and to achieve a NaCl concentration of 0.145 M, 20 Na⁺ ions and 17 Cl⁻ ions were added to the complex. A total of 3,000 steps of energy minimization were performed using the steepest descent method. Subsequently, the minimized system was equilibrated and run for 20 ps at a constant temperature of 300.00 K, followed by a 10 ns production run. Basic parameters for trajectory analysis such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), and hydrogen bond (H-bond) interactions were analyzed for each protein-ligand complex.

2.6. Structure-Affinity Relationship Study. Considering the generated information is relatively limited, investigation of the promising drug analogs will provide information for further study such as structure modification. After the in silico screening and the re-docking study, the analogs of the promising drug were collected by referring to literatures from PubMed, Elsevier, Springer, and Google Scholar. Then, one-by-one docking of the analogs targeting on Mpro was performed. Based on the analogs’ binding affinities, the structure-affinity relationship of the promising drug was summarized.

2.7. In Vitro Activity Assays of the SARS-CoV-2 Mpro Inhibitors. The inhibition rate of "old drug" on Mpro enzyme was measured using the 2019-nCoV Mpro inhibitor screening kit (P0312S, Beyotime Biotechnology, Shanghai, China) according to the manufacturer’s instructions. This kit was monitored at excitation of 340 nm and emission of 490 nm wavelengths on a microplate multimode reader by fluorescence resonance energy transfer. The percentage inhibition was calculated.

2.8. Literature Review of Promising Drug. After the in silico and in vitro study, we further questioned whether the promising "old drug" possessed documented biological activities associated with the pathological changes in COVID-19 condition. We searched PubMed, Elsevier, Springer, and Google Scholar for articles describing SARS-CoV-2 virus, COVID-19 condition, and the biological changes during the drug use. The condition of COVID-19 and the drug effect on pathological changes were summarized.

3. Results and Discussion

3.1. Pharmacophore of the Co-Crystal Ligand N3. As illustrated in Figure 1, Mpro monomer has three domains: domain I (6 antiparallel β-sheet), domain II (6 antiparallel β-sheet), and domain III (α-helices, closely related to proteolytic activity), and a long loop connects domains II and III. A highly conserved substrate-binding pocket (with a Cys145-His41 catalytic dyad) is located in a cleft between domains I and II, suggesting the antiviral inhibitors targeting this site should have a broad-spectrum anti-coronavirus activity [11].

As shown in Figure 1, a covalent bond between the S atom of Cys145 and the Cβ of the vinyl group is formed, supporting the critical Michael addition in the catalytic mechanism [5]. The lactam of P1 site inserts into the subsite S1 and forms a hydrogen bond with His163, while Leu of P2 site inserts deeply into a hydrophobic subsite. The Val of P3 site is exposed to solvent, tolerating a variety of functional group substitutions. The Ala of P4 is in a hydrophobic pocket. P5 site makes van der Waals interactions with Pro168, Thr190, and Ala191, while the aromatic ring forms van der Waals contacts with Thr24 and Thr25. N3 forms multiple hydrogen bonds with the active site residues, locking the inhibitor inside the binding pocket, which determines the inhibition of the enzyme as well as the coronavirus replication [11].

Mpro, which is highly conserved among all coronavirus, is a good target for the development of a single antiviral agent or in combination with other potential therapies to provide an effective first line of defense against all coronavirus-associated diseases [16]. The cocrystal structure of SARS-CoV-2 Mpro complexed with N3 is a good model for identifying inhibitor lead through in silico screening.

3.2. In Silico Screening by AutoDock Vina. For docking validation, N3 was re-docked into Mpro. The described docking workflow allowed top-ranked and reproduced binding conformation which was close to those of the 6LU7 co-crystal structure (checked by PyMOL, RMSD of 1.126 Å). In this study, a molecule with binding affinity ≤8.5 kcal/mol was treated to be potential based on recent reports on in silico screening of SARS-CoV-2 Mpro inhibitors [21].

All the 135 "old" drug structures, biological activities, targets, and top-ranked binding affinities were summarized (Supporting Information Table S1). In which, 6 molecules including anti-HIV drug (raltegravir), antibacterial drugs (cefonicid, cefoperazone, and minocycline), and antiadibiotics (canagliflozin and glyburide) showed high affinities (≤8.5 kcal/mol) as well as interesting binding conformations (bound to the Mpro active site and formed interesting interactions with key residues). In particular, the antibiotic minocycline, an inhibitor of bacterial ribosomal RNA, showed the highest binding affinity (~9.6 kcal/mol) compared to N3 (~7.7 kcal/mol). These small molecular drugs might be Mpro inhibitors of SARS-CoV-2.

Minocycline is a second-generation tetracycline antibiotic with an established safety profile that has been used in clinic for more than 30 years. It selectively binds to the 16S rRNA, inhibiting the binding of RNA to ribosomes, and interferes with protein synthesis [22]. The main treatment conditions of minocycline were Gram-positive/negative bacterial infections and the more recent multidrug-resistant Acinetobacter baumannii [23].
3.3. Re-Docking of N3 and Minocycline by Discovery Studio. To further validate the docking result, re-docking and comparison of docking results generated by different software are academically consensus. From the CDOCKER results generated by Discovery Studio, N3 (Figure 2(a)) formed conventional hydrogen bonds with residues Phe140, His163, His164, Glu166, Gln189, and Thr190. The isoxazole group formed pi-alkyl interaction with Ala191 and Pro168. The aromatic ring formed van der Waals' forces with residues Tr24, Tr25, Leu27, and Cys145. In addition, N3 molecule forms covalent bonds with multiple residues of Mpro. The docking results were closely consistent with the co-crystal structure (checked by PyMOL, RMSD of 1.650 Å).

For minocycline (Figure 2(b)), it contains multiple hydrophilic groups which formed conventional hydrogen bonding networks with key residues Phe140, Gly143, Cys145, His164, and Glu166 in the active site. The hydrophobic aromatic rings formed van der Waals’ forces with multiple amino acid residues of Mpro. It is commonly accepted that the covalent bond formed between the Cys145-His41 catalytic dyad and the designed compound would increase the Mpro inhibition potency, resembling the intermediate during substrate cleavage [5]. Beside the hydrogen bond between Cys145 and 2-carboxamide, a critical pi-cation formed between His41 and 4-dimethylamino group, which strongly supported minocycline as a Michael addition acceptor binding with the exact catalytic site to inhibit Mpro. These results indicated that the multiple especially critical interactions stabilized minocycline-Mpro in a low energy state, which was required for Mpro selection and antiviral activities.

3.4. MD Simulation. The ligand-binding status in the physiological milieu was predicted by MD simulation. The MD simulation of Mpro-N3 and Mpro-minocycline complexes was performed for 10 ns. RMSD is calculated considering the proteins’ backbone with respect to the initial conformations [24]. The RMSD values of the Mpro-N3 complex remained constant (~3 Å) from 0–4 ns and reached another plateau state after 5 ns and maintained the deviation below 2.5 Å. The RMSD value of Mpro-minocycline complex was found to be stable without significant deviation which was maintained below 3.5 Å (Figure 3(a)). These results preliminarily showed that there was no major deviation or conformation adjustment as the interaction of these ligands with the protein is stable. The RMSF was used for quantifying local changes/amino acid fluctuations along the protein chain [25]. Fluctuations for each of the individual amino acid of the target protein in case of N3 and minocycline were observed from the RMSF values. The average fluctuation of the amino acid residue for Mpro-N3 complex was 5.26 Å, and that was 6.85 Å for the Mpro-minocycline complex (Figure 3(b)). Less RMSF fluctuation indicates more interaction with the active site [26]. Subsequently, we investigated the persistence and variability of H-bond interactions by plotting H-bond thermograms. As shown in Figure 4, the H-bond interactions changed dynamically at different times throughout the simulation, but the total number of the interactions remained relatively stable. This indicated that the H-bond interactions in the Mpro-minocycline complex were highly persistent and stable.

3.5. Structure-Affinity Relationship of Minocycline. By referring to the literatures from PubMed, Elsevier, Springer, and Google Scholar, a database of minocycline analogs (44-compound, in which 21 compounds were clinical drugs) was established. After molecular docking, the chemical structures and top-ranked binding affinities of the analogs were summarized (Supporting Information Table. S2).

Indeed, minocycline showed a promising highest binding affinity among all the 44 analogs. Structures containing the main octahydrrotetracene-2-carboxamide skeleton were analyzed and the structure-affinity relationship was summarized (Figure 5). Carbonyl functional groups should be kept, and the middle hydroxyl group might be better if changed to carbonyl. Furthermore, the terminal 2-carboxamide could be modified with moderate (not too long) moiety. On the 4,7-bis(dimethylamino) side,
**Figure 2:** (a) Diagram of the interactions between N3 and M\textsuperscript{pro} and (b) diagram of the interactions between minocycline and M\textsuperscript{pro}. The key amino acid residue is shown in sphere, salt-bridge is shown in orange dashed line, conventional hydrogen bond is shown in green dashed line, carbon-hydrogen bond is shown in light blue dashed line, and pi-cation is shown in bright-orange dashed line.

**Figure 3:** Different stability-related parameters of docked complexes using M\textsuperscript{pro} obtained from MD analysis: (a) RMSD and (b) RMSF.
4-dimethylamino group is critical for the high affinity, and the S-stereochemistry of C4 is better than the R-stereochemistry, which was also verified from the previous binding mode investigation that it could form the key covalent bond with His41.

Docking simulation and the structure-affinity relationship study revealed that critical covalent bond formed between minocycline and the Cys145-His41 catalytic dyad of Mpro, which helped us to better understand why the functional groups as well as the tetracycline skeleton could be suitable for the Mpro active site binding.

3.6. In Vitro Activity Assays of the SARS-CoV-2 Mpro Inhibition. As shown in Figure 6, minocycline showed Mpro inhibitory activity in a dose-dependent manner with IC50 of 5 mM. The in vitro validation result suggests that the inhibitory activity against SARS-CoV-2 Mpro of minocycline might be beneficial in addition to other well-known mechanisms. Furthermore, minocycline could be used as an interesting lead to design analogs that can more potently and selectively inhibit SARS-CoV-2 Mpro to improve its antiviral activity and avoid the unwanted adverse effects associated with other mechanisms.

Minocycline has been used in pharmacological conditions of both bacterial and mycoplasma infections. Moreover, minocycline appears to have broad-spectrum antiviral activities: reducing West Nile virus titers in brain-derived cell types, reducing Japanese encephalitis-induced damage in neuronal cells, inhibiting H7N9 replication in human lung epithelial cells, and attenuating pathogenic immune responses during infection with human and simian immunodeficiency virus (HIV/SIV) [27–31]. Based on molecular docking and dynamic studies, minocycline was proposed as potential antiviral therapy against Congo Crimean hemorrhagic fever virus to inhibit the binding of virus to host nucleoprotein [32].

In a randomized controlled trial of dengue hemorrhagic fever patients, compared with standard-of-care, combination therapy with doxycycline (analog of minocycline) significantly decreased the TNF and IL-6 levels and mortality [33]. Tetracycline inhibiting proinflammatory cytokines and matrix metalloproteinases plays a key role in coronavirus acute infection and is involved in chemokine activation and in tissue destruction [34, 35]. Of note, this immunomodulatory effect seems to be dsRNA-mediated [36].

3.7. Literature Review of Minocycline. From PubMed, Elsevier, Springer, and Google Scholar databases, articles describing COVID-19 and minocycline use until 2022, June 1st, were searched. More than 10,000 papers including published and preprints were found, in which 1430 papers clearly clarified the biochemical indexes of COVID-19 patients, and 706 papers were associated with the anti-inflammatory effect of minocycline. The effect of COVID-19 conditions and minocycline on selected biomarkers including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), lactose dehydrogenase (LD), D-dimer, CD4+ T cell, CD8+ T cell, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-6, and IL-10 were summarized and analyzed (Table 1).

A variety of clinical data revealed that the inflammatory factor storm (IFS) existed and played a critical role in severe or fast-progressive COVID-19 condition. In the later phase, the level of ESR, CRP, IL-6, and D-dimer increased and the counts of lymphocytes, total T cells, CD4+ T cells, and CD8+ T cells were dramatically reduced, while patients in declining period presenting decreased levels of TNF-α, IFN-γ, IL-6, and IL-10 and restored counts of T cell [21, 63, 64, 85]. In severe COVID-19 patients, the elevation of the CRP level and white blood cell count might be accompanying with bacterial infection, and antibiotics were usually prescribed [49]. As shown in Table 1, the levels of
Figure 5: Primary structure-affinity relationship illustration of minocycline targeting on M\textsuperscript{pro}.

Figure 6: In vitro validation of the inhibitory activity of minocycline on M\textsuperscript{pro}.

Table 1: Comparison of the effect of COVID-19 and minocycline on selected biomarkers.

<table>
<thead>
<tr>
<th>Biological markers</th>
<th>COVID-19</th>
<th>Minocycline effect on physical or infectious challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe (references)</td>
<td>Nonsevere (references)</td>
</tr>
<tr>
<td>ESR</td>
<td>↑[37–40]</td>
<td>↑[37, 39, 41–44]</td>
</tr>
<tr>
<td>CRP</td>
<td>↑[37, 38, 48–53]</td>
<td>↑[37, 38, 43, 49, 50, 52, 54]</td>
</tr>
<tr>
<td>LD</td>
<td>↑[37, 51, 59, 60]</td>
<td>↑[37]</td>
</tr>
<tr>
<td>D-dimer</td>
<td>↑[37, 40, 50, 51, 59]</td>
<td>↑[37, 42, 43, 51, 54, 61]</td>
</tr>
<tr>
<td>CD\textsuperscript{4+} T cell</td>
<td>↑[63–65]</td>
<td>↑[39, 64, 66]</td>
</tr>
<tr>
<td>CD\textsuperscript{8+} T cell</td>
<td>↑[63, 64, 67]</td>
<td>↑[39, 64, 68]</td>
</tr>
<tr>
<td>TNF-\alpha</td>
<td>↑[63, 70]</td>
<td>↑[68, 71, 72]</td>
</tr>
<tr>
<td>IFN-\gamma</td>
<td>↑[63, 64]</td>
<td>↑[42, 64, 82]</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑[37, 40, 50, 63, 64, 82]</td>
<td>↑[37, 39, 44, 64, 66, 83]</td>
</tr>
<tr>
<td>IL-10</td>
<td>↑[63, 64]</td>
<td>↑[39, 64, 68, 72]</td>
</tr>
</tbody>
</table>

Note. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; LD: lactose dehydrogenase; TNF-\alpha: tumor necrosis factor \alpha; IFN-\gamma: interferon \gamma; IL-\: interleukin; ↑: significantly increased; ↓: significantly decreased.
Inflammatory responses triggered by viral infection play a crucial role in pulmonary pathology severity, suppressing the INS to reduce lung inflammation is a valuable treatment method [86]. High doses of glucocorticoid were widely applied during the outbreaks of SARS to suppress lung inflammation and immune response; however, it appeared to be associated with side effects [87–89]. Thalidomide (immunomodulatory and anti-inflammatory agent) in combination with antiviral drugs and low-dose glucocorticoid was reported the protective effect on lung injury and immunological stress caused by COVID-19 [90]. Chloroquine was included in the 6th version of Diagnostic and Treatment Protocol for COVID-19 in China due to the in vitro anti-SARS-CoV-2 results and in vivo anti-inflammatory activity. Hydroxychloroquine, which is chemically and biologically similar but safer than chloroquine, had been included in the local diagnostic and treatment guidelines for COVID-19 (Shanghai, China) [91]. In a two-year randomized controlled trial on early seropositive rheumatoid arthritis patients, minocycline achieved better anti-inflammatory outcomes than hydroxychloroquine [60]. The immune imbalance and bacterial infection often appear in the later stages of COVID-19; the efficacy of antiviral drugs might remain unsatisfactory [92]. The antibiotics and glucocorticoid were sometimes administered according to the clinical characteristics and physicians’ discretion [59].

Moreover, minocycline was reported to attenuate T cell and microglia activity to impair cytokine production in T cell-microglia interaction [78]. Angiotensin converting enzyme 2 (ACE2), the function receptor for SARS-CoV-2, is present in multiple human organs [93]. Cytokine release leads to serious complications in the cardiovascular system, digestive system, and central nervous system (CNS) in COVID-19 patients [48]. Because of its high lipophilicity and small size, minocycline can cross the blood-brain barrier and accumulate in cerebrospinal fluid (CSF) and CNS cells, resulting in beneficial effects on CNS diseases.

Besides, recent evidence suggested that the precise site of interaction between minocycline and cellular RNA molecules could be double-stranded RNAs (dsRNAs), which have been observed as intermediates of the viral replication of positive-stranded viruses, the aberrant induction of inflammatory cytokines/chemokines in case of SARS infection was mostly activated by dsRNA intermediates [36, 94]. In addition, the robust viral replication and delayed IFN-γ signaling accompanying the initial steps of SARS seem to be consequence of the coronavirus ability to initially evade the host dsRNA sensors [95, 96]. Therefore, early administration of dsRNA-binding minocycline might reduce the risk of SARS-CoV-2.

4. What Is New and Conclusion

In conclusion, from in silico screening of 135 clinical drugs targeting on SARS-CoV-2 M^Pro^, minocycline, inhibitor of bacterial ribosomal rRNA, showed interesting binding affinity (~9.6 kcal/mol). Critical hydrogen bonding with the Cys145-His41 catalytic dyad and hydrophobic interactions were found between minocycline and M^Pro^ active sites. During the 0–10 ns MD simulations, molecular dynamics stability of M^Pro^-minocycline complex was also demonstrated to be close to M^Pro^-N3 complex with persistent H-bond interactions. The structure-affinity relationship explained the conformational suitability of minocycline. Minocycline showed in vitro M^Pro^ inhibitory activity with IC_{50} of 5 mM. Literature review found that minocycline had both in vitro and in vivo broad-spectrum antiviral as well as anti-inflammatory activities, and the levels of a broad spectrum of biological markers during minocycline administration were opposed to those of COVID-19 condition. These findings suggested that minocycline, a safe, inexpensive, and readily available antibiotic, could be considered as an adjunctive therapeutic option for severe and fast-progressive COVID-19 patients. This study shed a new light on an adjuvant treatment strategy for this viral disease. Limitations of this study include the short duration of the MD simulations, which were performed for only 10 ns, resulting in only a brief understanding of the conformational changes of M^Pro^-minocycline complex.

Data Availability

The results of in silico screening of SARS-CoV-2 M^Pro^ inhibition from old drugs and the results of in silico screening of minocycline analogs used to support the findings of this study are included within the supplementary information files.

Disclosure

A previously published preprint is available at https://www.researchsquare.com/article/rs-1528733/v1 [97].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Professors Guanhua Du and Zhanfei She conceptualized and supervised this study. Guanhua Du and Bin Xiao designed the research. Bin Xiao, Xianxiang Bai, Liwen Ren, and Jinhua Wang performed virtual screening and analyzed the docking results’ analysis. Yaru Han and Si Wu designed and carried out the literature review. Yaru Han and Xiurong Luan performed the in vitro M^Pro^ inhibitory assay. All authors revised the manuscript and have read and approved the final manuscript. Yaru Han, Xinxiang Bai, and Su Wu contributed equally to this work.

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**Supplementary Materials**

Table S1: Results of in silico screening of SARS-CoV-2 main protease (Mpro) inhibition from old drugs. Table S2: Results of in silico screening of minocycline analogs. *(Supplementary Materials)*

**References**


