

Prediction of potential 3CL^{pro}-targeting anti-SARS-CoV-2 compounds from Chinese medicine

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Abstract

The recent outbreak of coronavirus disease 2019 caused by the new coronavirus, SARS-CoV-2, has become an international emergency. Since there is no effective therapy for the treatment of this disease, drugs or vaccine that can prevent or cure the SARS-CoV-2 infection are urgently needed. The viral 3-chymotrypsin-like cysteine protease (3CL^{pro}), which plays a key role in the replication of coronavirus, is a potential drug target for the development of anti-SARS-CoV-2 drugs. With the crystal structure of 3CL^{pro}, we performed virtual screening from a small chemical library of a Traditional Chinese Medicine recipe- FuFang Zhenzhu Tiaozhi (FTZ). Five compounds with the

best scores were screened and could be considered as potential hit compounds to be investigated further with bioassays for their anti-virus effects.

Keywords: SARS-CoV-2, COVID-19, Molecular docking, Chinese medicine, lithospermic acid B, specnuezhenide, neonuezhenide, rutin, neodiosmin.

1. Introduction

In December 2019, a new coronavirus, SARS-CoV-2 was reported to cause pneumonia illness in Wuhan City, Hubei Province, China. In January 2020, this coronavirus disease 2019 (COVID-19) was confirmed human-to-human transmission and spread in China [1]. The World Health Organization (WHO) has declared this a Public Health Emergency of International Concern (PHEIC) on 31 January 2020. Until now, there are 81,024 confirmed cases reported in mainland China, with 3181 deaths. The WHO reported that there are more than one hundred thousand confirm cases all over the world.

Apart from China, the situation is very serious in Italy, Korea and Iran. Therefore, there is an urgent need to discover new drug or vaccine to prevent or cure the COVID-19.

3CL protease ($3CL^{Pro}$, also known as M^{Pro}) is the main protease produced by the coronavirus and plays a key role in the virus' replication. Most of the functional proteins of coronaviruses are encoded by specific genes, which are first translated into polyproteins and then cleave by $3CL^{Pro}$ into multiple proteins with different activities for virus replication [2-4]. $3CL^{Pro}$ is therefore considered to be a potential drug target

against COVID-19.

Traditional Chinese Medicine (TCM) has been used to prevent pestilence in China for thousands of years [5]. In the treatment of SARS, TCM was confirmed to reduce hormone consumption, alleviate fever symptoms and complications effectively [6]. A retrospective analysis of COVID-19 patients newly published in the Lancet showed that 8 of the 41 infected patients had diabetes mellitus, suggesting that patients with diabetes are likely to be highly susceptible to the SARS-CoV-2 [7]. Our research group has focused on the treatment of glycolipid metabolic disease for decades and has developed a prescription called FuFang Zhenzhu Tiaozhi (FTZ) [8], which can effectively regulate glycolipid metabolic disorder. Since the development of a new drug or vaccine to fight SARS-CoV-2 could be time-consuming, screening active compounds from TCM targeting 3CL^{Pro} could be a potential strategy for treating COVID-19. In the present study, we used molecular docking to screen bioactive molecules from FTZ against 3CL^{Pro} and five natural compounds including lithospermic acid B, specnuezhenide, neonuezhenide, rutin and neodiosmin (Figure 1) were predicted to target 3CL^{Pro}.

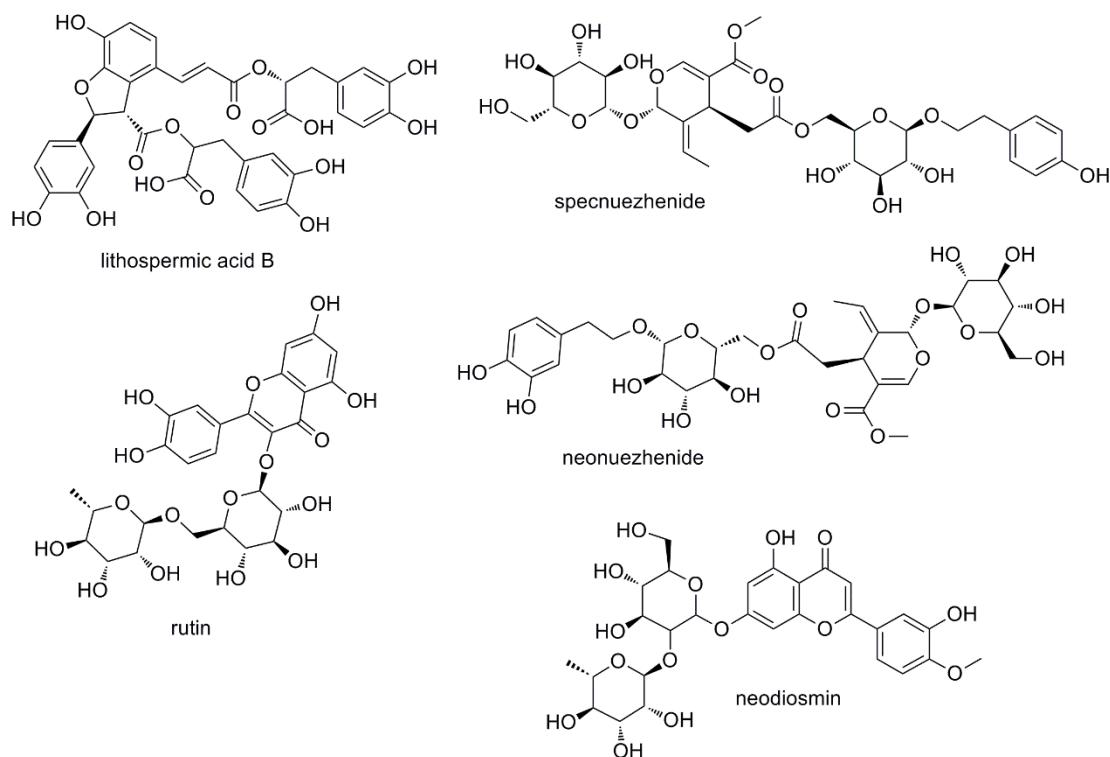


Figure 1. Chemical structures of compounds screened out by docking: lithospermic acid B, specnuezhenide, neonuezhenide, rutin and neodiosmin.

2. Result and Discussion

2.1. Lithospermic acid B

Lithospermic acid B is one of polyhydroxy phenolic acid compounds isolated from *Salvia miltiorrhiza* (Chinese name: Dan shen). This bioactive compound has many pharmacological activities, such as improving renal function, preventing and curing cardiovascular disease, anti-oxidation, anti-fibrosis, anti-inflammatory and so on [9].

The docking results revealed that lithospermic acid B can bind into the binding pocket of with a CDOCKER interaction energy of -76.76 kcal/mol. A number of conventional hydrogen bond interactions were predicted to be established between lithospermic acid B and the side chains of THR26, PHE140, ASN142, GLY143 and GLU166. Moreover,

THR25 and ASP187 interact with lithospermic acid B via carbon hydrogen bond. MET165 can form hydrophobic interaction with lithospermic acid B. Moreover, there are 16 amino acids (such as LEU27, HIS41, MET49, etc.) of 3CL^{Pro} can form van der Waals force with lithospermic acid B (Figure 2). *Salvia miltiorrhiza* has been used in China alone or combined with other Chinese herbal medicines for many years without any significant adverse effects reported. Based on the relative low toxicity of lithospermic acid B and its potential binding to 3CL^{Pro}, lithospermic acid B may be one of the potential candidates for COVID-19 treatment.

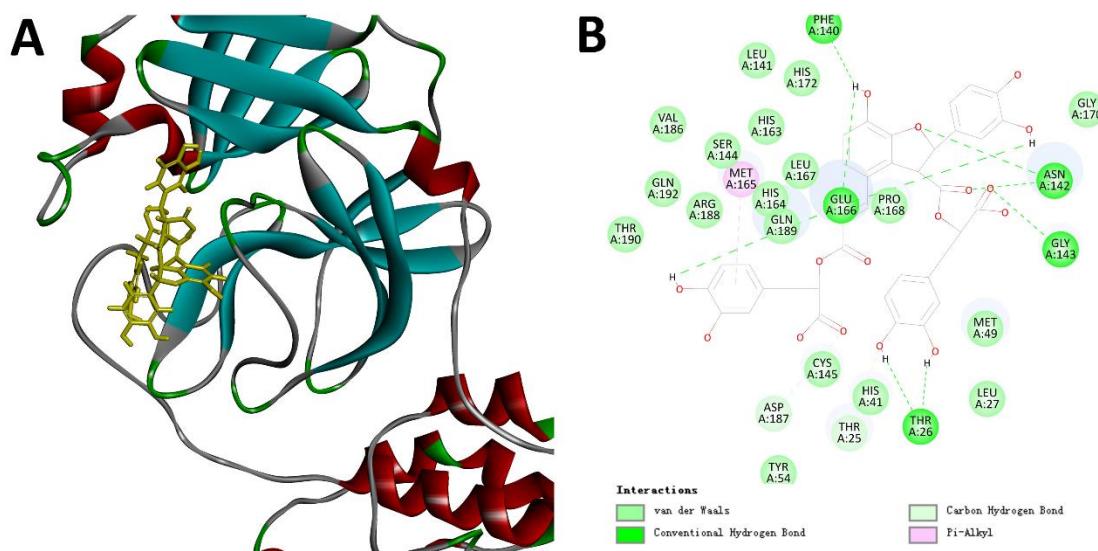


Figure 2. (A) Docking pose of lithospermic acid B; (B) The predicted interactions from lithospermic acid B and the amino acids residues of 3CL^{Pro}.

2.2. Specnuezhenide and Neonuezhenide

Specnuezhenide and neonuezhenide are bioactive compounds from the fruits of *Ligustrum lucidum* and belong to secoiridiod glycosides. These two compounds have similar binding pose with 3CL^{Pro} (Figure 3A). The CDOCKER interaction energy of specnuezhenide and neonuezhenide against the protein are -70.78 and -67.23 kcal/mol,

respectively. The 2D ligand interaction diagram (Figure 3B) shows the predicted interactions between specnuezhenide and the 3CL^{Pro} residues. The binding of specnuezhenide with THR26, GLY143, MET165, GLU166 and GLN189 of 3CL^{Pro} protein are through conventional hydrogen bonds. Carbon hydrogen bonds can be found between THR25/PHE140/LEU141/GLY143/GLU166/GLN189 and specnuezhenide. In addition, specnuezhenide can also interact with HIS41, CYS145 and HIS172 through hydrophobic interaction. Moreover, van der Waals force is found for specnuezhenide interacting with a few amino acids such as SER46, SER144 and PRO168 around the binding pocket. Since the molecular structure of neonuezhenide is very similar to specnuezhenide, the predicted interactions between neonuezhenide and 3CL^{Pro} residues are comparable with specnuezhenide (Figure 3C). Currently, there are only a few pharmacological studies on these two compounds. Previous study reported that secoiridiod glycosides have hypoglycemic, anti-inflammatory, anti-tumor effects [10-11]. Since *Ligustrum lucidum* has been used as TCM for a long time, it could be worth to test whether specnuezhenide and neonuezhenide show inhibitory activity against the virus targeting 3CL^{Pro} protein.

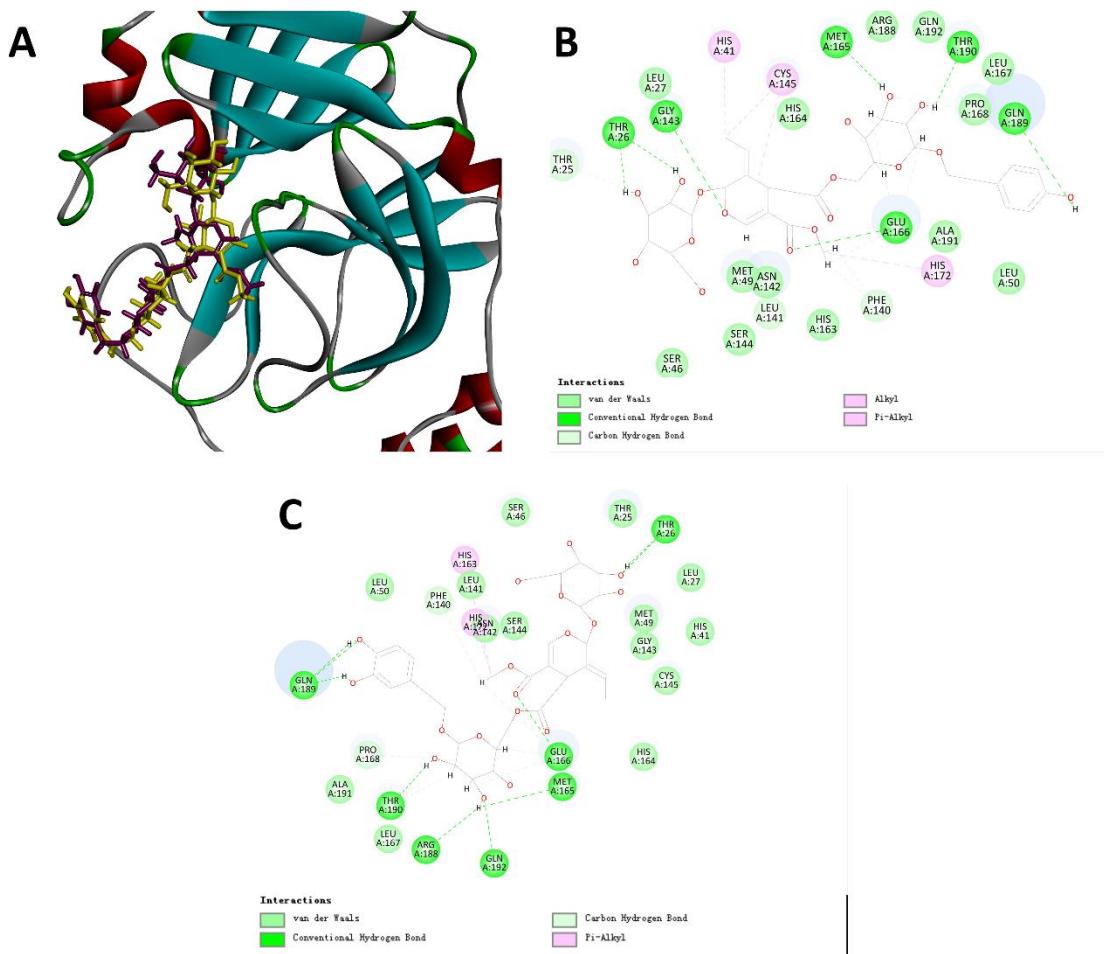


Figure 3. (A) Docking pose of specnuezhenide (yellow) and neonuezhenide (purple); (B) The predicted interactions from specnuezhenide and the amino acids residues of 3CL^{Pro}; (C) The predicted interactions from neonuezhenide and the amino acids residues of 3CL^{Pro}.

2.3. Rutin

Rutin is also known as quercetin-3-O-rutinoside and is a flavonol glycoside widely found in plants. It has been found to have antioxidant, anti-inflammatory and anti-free radical effects [12]. Our docking results suggested that rutin also targets 3CL^{Pro} with a CDOCKER interaction energy of -69.38 kcal/mol. The 2D interaction diagram (Figure 4B) shows that the flavonol groups of rutin formed hydrophobic bonds with

surrounding amino acid residues (MET49 and CYS145) as a key force to grasp the 3CL^{Pro} protein. Moreover, the hydroxyl groups of rutin interact with THR26, LEU141, GLU166 and ASP187 through conventional hydrogen bonds. In addition, the carbonyl group of rutin can form carbon hydrogen bond with MET165 and GLU166 residues. Rutin can also form van der Waals interactions with 17 amino acid residues such as THR25, LEU27, TYR54 and THR190. Based on the prediction, we hypothesize that rutin is possibly active to inhibit SARS-CoV-2 through inhibiting 3CL^{Pro}. However, it needs further investigation with *in vivo* assays.

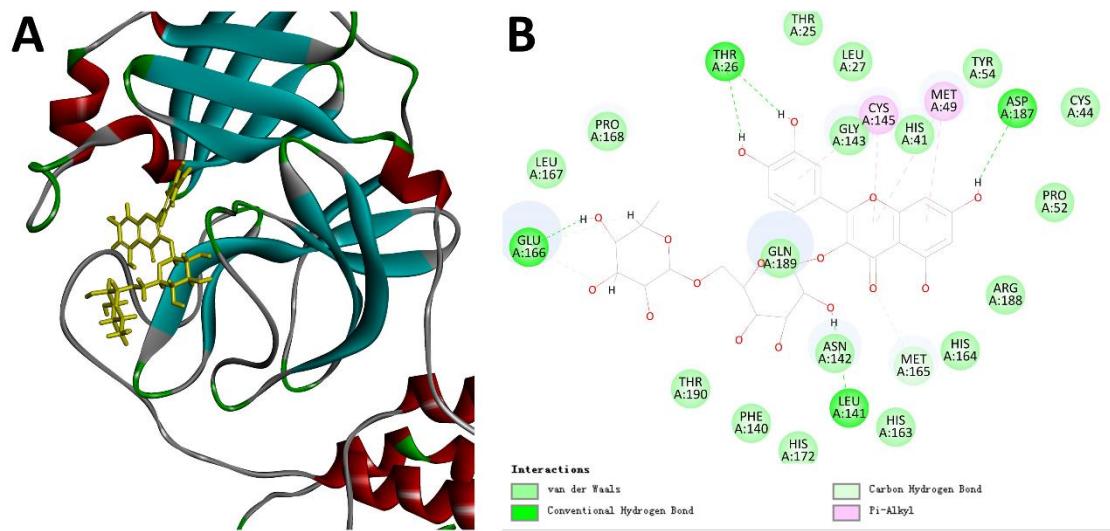


Figure 4. (A) Docking pose of rutin; (B) The predicted interactions from rutin and the amino acids residues of 3CL^{Pro}.

2.4. Neodiosmin

Neodiosmin is a flavonoid glycoside isolated from limes and it can also be produced by the dehydrogenation of neohesperidin. Neodymium is mainly used to inhibit the bitter taste produced by naringin and limononin in the industrial production of orange juice [13]. So far, no report about its pharmacological activity has been found in the

literature. The CDOCKER interaction energy of neodymium to 3CL^{Pro} protein was predicted to be -67.27 kcal/mol. The docking results showed that six hydrogen bonds can be formed between neodiosmin and the amino acids HIS41, LEU141, GLY143, HIS163 and THR190 of 3CL^{Pro} protein. HIS41, LEU141, ASN142 and ASP187 can interact with neodiosmin through carbon hydrogen bond. Moreover, HIS41, MET49 and MET165 can form hydrophobic interactions with neodiosmin. In addition, neodymium can form van der Waals force interactions with 14 amino acids of the protein (Figure 5B). The results may suggest that neodiosmin may bind to 3CL^{Pro} and possibly affect the activity of SARS-CoV-2.

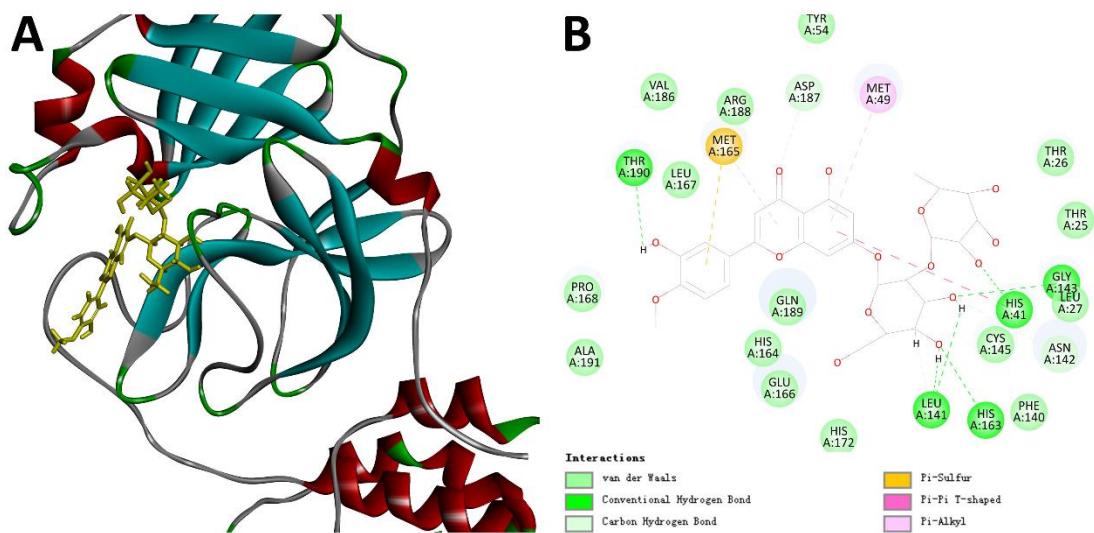


Figure 5. (A) Docking pose of neodiosmin; (B) The predicted interactions from neodiosmin and the amino acids residues of 3CL^{Pro}.

3. Summary

Since the SARS-CoV-2 is spreading at a rate and scale much worse than previous coronaviral epidemics, there is an urgent need to find a feasible way to fight against COVID-19. In this study, five compounds including lithospermic acid B,

specnuezhenide, neonuezhenide, rutin and neodiosmin were predicted to bind into the binding pocket of 3CL^{Pro} protease through virtual screening from our small TCM chemical library. Molecular docking suggests that these compounds could have effective interacts with the protein through stable hydrogen bonds and hydrophobic interactions. Based on the results, we propose for the first time that lithospermic acid B, specnuezhenide, neonuezhenide, rutin and neodiosmin could potentially inhibit the biological function of 3CL^{Pro} protease to achieve anti-SARS-CoV-2. Further investigation is needed to confirm their binding affinity with 3CL^{Pro} and antivirus effects on SARS-CoV-2.

4. Methods

Discovery Studio (DS) 2016 was used to perform the molecular modeling study. The X-ray crystal structure of 3CL^{Pro} was downloaded from the PDB database (PDB entry: 6LU7) [14]. This structure is in complex with a SARS-CoV inhibitor, N3. Since the identity of 3CL^{Pro} between SARS-CoV-2 and SARS-CoV is 96%, it is feasible to screening anti-SARS-CoV-2 compounds with N3 binding pocket [4]. The protein and the ligands were prepared for this study using the own tools in DS. And then, the CDocker protocol was used to predict the binding poses with default parameters [15]. The top 3% scoring poses were containing 21 docking poses, and these poses were generated by five compounds. The top-scoring pose for each of the five compounds was selected to visually inspect.

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Conflict of Interest

The authors declare no competing interests.

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