Potential therapeutic approaches of Traditional Chinese and Western Medicine targeting nsp14 of 2019-nCoV

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Abstract

In December 2019, a severe outbreak of novel coronavirus (2019-nCoV) infection emerged in Wuhan, China, creating an urgent need for therapeutic agents against 2019-nCoV. Coronavirus nonstructural proteins (NSPs) play essential roles in viral genome replication and transcription, are broadly conserved across the coronavirus family, and constitute important functional proteins of coronaviruses. Among them, the NSP14 protein possesses both exoribonuclease and methyltransferase activities, representing a critical target for anti-SARS and anti-coronavirus drug development. Sequence analysis demonstrated that the SARS coronavirus (PDB ID: 5nfy) exhibits 98.7% amino acid sequence homology with the novel coronavirus isolate from the Wuhan Huanan Seafood Wholesale Market (GenBank: MN985325.1). In this study, homology modeling was performed using the published SARS-NSP14 crystal structure (PDB ID: 5nfy) to construct a three-dimensional structural model of the 2019-nCoV-NSP14 protein. Virtual screening was conducted separately against its N-terminal methyltransferase and C-terminal methyltransferase structural domains, yielding 18 compounds with inhibitory activity against NSP14. Among these, saquinavir, bromocriptine, baicalein, and hypericin were identified as capable of simultaneously interacting with both aforementioned functional domains of the NSP14 protein. These findings suggest that the established anti-HIV drug saquinavir, along with the key antiviral active components from traditional Chinese medicine—baicalein and hypericin—may exert antiviral effects by targeting the 2019-nCoV-NSP14 protein, and thus warrant further investigation as candidate therapeutics against 2019-nCoV.

Full Text

Preamble

Potential Treatment of Chinese and Western Medicine Targeting nsp14 of 2019-nCoV

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Abstract

The 2019 novel coronavirus (2019-nCoV) caused a severe, large-scale acute respiratory disease outbreak in Wuhan, China, and subsequently spread to other regions and countries worldwide, posing a serious threat to human health. There is an urgent need to develop drugs for the prevention and treatment of 2019-nCoV. The 2019-nCoV nonstructural protein 14 (NSP14), which carries RNA cap guanine N7-methyltransferase and 3' -5' exoribonuclease activities, could serve as a potential drug target for therapeutic intervention. NSP14 of 2019-nCoV shares 98.7% sequence similarity with its counterpart from severe acute respiratory syndrome (SARS) coronavirus (PDB ID: 5nfy). Using the SARS NSP14 structure as a template, we modeled the 2019-nCoV NSP14 structures for virtual screening. Based on docking scores, 18 small-molecule drugs were selected for further evaluation. Notably, compounds including Saquinavir, Hypericin, Baicalein, and Bromocriptine could bind to both the N-terminus and C-terminus of the 2019-nCoV NSP14 homology model, thus representing candidate drugs against 2019-nCoV warranting further investigation.

1. Introduction

In December 2019, a large-scale outbreak of severe acute respiratory disease caused by the "2019 novel coronavirus (2019-nCoV)" occurred in Wuhan, China, spreading to other regions of China and countries worldwide within the following month and seriously threatening human health. This has created an urgent demand for developing effective drugs for the prevention and treatment of 2019-nCoV. Coronavirus nonstructural proteins (NSPs) play crucial roles in viral genome replication and transcription and are generally conserved as functionally important proteins across the coronavirus family. Among these, the NSP14 protein possesses both exonuclease and methyltransferase functions that are essential for replication and transcription in SARS and other coronaviruses, making it an attractive target for drug design.

Amino acid sequence alignment revealed that 2019-nCoV NSP14 shares 98.7%

similarity with SARS NSP14 (PDB ID: 5nfy). [Figure 1: see original paper] Consequently, we modeled the 2019-nCoV NSP14 structure using SARS NSP14 (PDB ID: 5nfy) as a template. The N-terminus and C-terminus of 2019-nCoV NSP14 were designated as active sites for drug screening. A total of 7,496 drugs obtained from the ZINC database were subjected to binding screening, comprising 2,100 FDA-approved drugs, 4,264 drugs approved by other regulatory agencies (world-not-FDA), and 1,132 investigational drugs undergoing clinical trials but not yet approved. Docking was performed using AutoDock Vina 1.1.2. Ten top compounds with the lowest negative Vina scores (ranging from -8.6 to -9.7 kcal/mol) were selected from the N-terminal domain of the homology model , while eight top compounds with the lowest negative Vina scores (ranging from -8.7 to -9.7 kcal/mol) were identified from the C-terminal domain . Importantly, compounds including Saquinavir, Hypericin, Baicalein, and Bromocriptine could bind to both the N-terminus and C-terminus of the homology model and occupy the corresponding active pockets of 2019-nCoV NSP14.

2.1 Docking Method

The SARS NSP14 amino acid sequence was downloaded from the PDB protein structure database (PDB ID: 5nfy), and the 2019-nCoV amino acid sequence (Accession number: MN908947) was obtained from the National Center for Biotechnology Information (NCBI) database. The homology of these amino acid sequences was aligned using ClustalW. The homology model of the target protein was constructed and optimized using Modeller 9.18, with the crystal structure of SARS NSP14 (PDB ID: 5nfy) serving as the template. A total of 100 independent structures were generated, and the one with the best DOPE score was selected for further energy minimization using Amber. Ligands were downloaded from the ZINC database (FDA, world-not-FDA, and investigational-only subsets; http://zinc.docking.org/substances/subsets/). The 2D structures of the compounds were converted into corresponding 3D coordinates using the Babel server (http://openbabel.sf.net). The protein model was converted to pdbqt format using the prepare_{receptor4}.py script with assigned atom types and partial charges. All rotatable bonds in the ligands were set as flexible for flexible docking. Vina 1.1.2 was employed for molecular docking, with docking boxes selected at the N-terminal exonuclease domain (aa: 62-290) and C-terminal methyltransferase domain (aa: 291-527) of 2019-nCoV NSP14, respectively.

2.2 Binding Free Energy Calculation

Each simulation system was immersed in a cubic TIP3P water box with a 10 Å distance from the solute, and Na^+ or Cl^- ions were added to neutralize the system. The General Amber Force Field (GAFF) and Amber ff14SB force field were used to parameterize the ligands and protein, respectively. Each system underwent 10,000 steps of minimization with constraints (10 kcal/mol/$Å^2$) on heavy atoms of the complex, including 5,000 steps of steepest descent minimiza-

tion followed by 5,000 steps of conjugate gradient minimization. Subsequently, each system was heated to 300 K over 0.2 ns and equilibrated for 0.1 ns in the NPT ensemble. Finally, 5 ns MD simulations were performed on each system at 300 K. The minimization, heating, and equilibration steps were carried out using the sander program in Amber18, while the 5 ns production run was performed with pmemd.cuda. Based on the 5 ns MD simulation trajectories, binding free energy (ΔG) was calculated using the MM/GBSA method according to the following equation:

$$\Delta G_{\text{cal}} = \Delta H - T\Delta S = \Delta E_{\text{vdw}} + \Delta E_{\text{ele}} + \Delta G_{\text{gb}} + \Delta G_{\text{np}} - T\Delta S$$

where ΔE_{ele} and ΔE_{vdw} refer to electrostatic and van der Waals energy terms, respectively, and ΔG_{gb} and ΔG_{np} refer to polar and nonpolar solvation free energies, respectively. Conformational entropy ($T\Delta S$) was not calculated to save computational time; however, since the ligands were compared against the same target, ignoring entropy is reasonable for relative binding affinity assessment.

3.1 Docking Results of Saquinavir Against 2019-nCoV NSP14 Model

Saquinavir, the first FDA-approved HIV protease inhibitor, has been used to treat patients with human immunodeficiency virus (HIV) infection since 1995. Our docking results showed that five hydrogen bonds involving Asp-273, Asn-252, Asp-90, and Leu-253 were maintained upon Saquinavir binding to the N-terminus of 2019-nCoV NSP14 (Fig. 1A). Meanwhile, hydrogen bonds involving Asn-386, Gln-313, Gly-333, and Thr-428 were maintained upon Saquinavir binding to the C-terminus (Fig. 1C). Saquinavir could bind to both the N- and C-terminal active pockets of 2019-nCoV NSP14 (Fig. 1B, D). A recent study using a drug-target interaction deep learning model demonstrated that Saquinavir can bind to 2019-nCoV RNA-dependent RNA polymerase and inhibit its enzyme activity. Our simulation results further indicate that Saquinavir can bind to two active sites of NSP14, suggesting it could serve as a candidate drug against 2019-nCoV for further research.

3.2 Docking Results of Hypericin Against 2019-nCoV NSP14 Model

Hypericin, a main ingredient in the traditional Chinese medicine *Hypericum perforatum* L. (St. John' s wort), has demonstrated activity against RNA viruses in vitro by inhibiting viral replication. The present docking results show that three hydrogen bonds involving Asn-252, Gly-93, and His-268 were maintained upon Hypericin binding to the N-terminus of 2019-nCoV NSP14 (Fig. 2A). [Figure 2: see original paper] While six hydrogen bonds involving Asn-306, Arg-310, Asn-422, and Lys-336 were maintained upon Hypericin binding to the C-terminus (Fig. 2C). Hypericin can bind to both the N- and C-terminal active pockets of

2019-nCoV NSP14 (Fig. 2B, D). Hypericin has been proven to exert inhibitory effects on human hepatitis C virus (HCV) and human immunodeficiency virus (HIV). Combined with the present findings, Hypericin may possess potential antiviral activity against 2019-nCoV. The traditional Chinese medicine *Hypericum perforatum* L., a main component of Shuanghuanglian oral liquid, has been widely used for treating viral influenza. However, the suggestion that Shuanghuanglian oral solution could treat 2019-nCoV triggered a major public trust crisis among Chinese scientists. We propose that the anti-2019-nCoV effects of Hypericin should be tested in 2019-nCoV infection cell culture models to better understand whether Chinese medicines represent a viable treatment approach for 2019-nCoV.

3.3 Docking Results of Baicalein Against 2019-nCoV NSP14 Model

Baicalein, a flavonoid compound isolated from the root of *Scutellaria baicalensis* Georgi (Huang Qin in Chinese), inhibits viral replication of parainfluenza virus, influenza A virus, hepatitis B virus, HIV-1, and SARS coronavirus. The present docking results showed that six hydrogen bonds involving Asn-266, Asp-273, Gly-93, Glu-92, and His-268 were maintained upon Baicalein binding to the N-terminus of 2019-nCoV NSP14 (Fig. 2A), while four hydrogen bonds involving Asn-386, Asp-331, and Gln-313 were maintained upon Baicalein binding to the C-terminus (Fig. 2C). Baicalein can also bind to both the N- and C-terminal active pockets of 2019-nCoV NSP14 (Fig. 2B, D). Previous studies demonstrated that Baicalein acts as a novel chemical inhibitor of the ATPase activity of SARS coronavirus NSP13 protein. The present data suggest that Baicalein may bind to NSP14 protein to exert anti-2019-nCoV activity. Therefore, we believe the anti-2019-nCoV activity induced by Baicalein warrants further investigation.

3.4 Docking Results of Bromocriptine Against 2019-nCoV NSP14 Model

Bromocriptine, a specific dopamine receptor agonist acting on the hypothalamus and pituitary, exhibits inhibitory effects on Dengue virus replication with low cytotoxicity (half-maximal effective concentration, EC_{50} = 0.8–1.6 M; half-maximal cytotoxicity concentration, CC_{50} = 53.6 M). Moreover, Bromocriptine inhibits Zika virus protease activity and shows synergistic effects with interferon-$ $2b against Zika virus replication. Interestingly, our molecular docking results reveal that Bromocriptine can bind to both the N- and C-terminal active pockets of 2019-nCoV NSP14 (Fig. 4B). [Figure 4: see original paper] The results showed that three hydrogen bonds involving Asn-104, Asp-273, and Gln-145 were maintained upon Bromocriptine binding to the N-terminus of 2019-nCoV NSP14 (Fig. 4A), while one bond involving Thr-428 was maintained upon Bromocriptine binding to the C-terminus (Fig. 4C).

3.5 Calculation of Binding Free Energy

Based on the 5 ns MD simulation trajectories, binding free energy (ΔG) was calculated using the MM/GBSA method. The calculated binding free energies of Saquinavir, Hypericin, Baicalein, and Bromocriptine for the N-terminus of the homology model were -37.2711 ± 3.2160, -30.1746 ± 3.1914, -23.8953 ± 4.4800, and -34.1350 ± 4.3683 kcal/mol, respectively . In contrast, the calculated binding free energies for C-terminus binding were -60.2757 ± 4.7708, -30.9955 ± 2.9975, -46.3099 ± 3.5689, and -59.8104 ± 3.5389 kcal/mol, respectively . Taken together, these results demonstrate that Saquinavir exhibits the strongest binding free energy among the tested compounds.

4. Conclusion

2019-nCoV NSP14 is a bifunctional enzyme carrying RNA cap guanine N7-methyltransferase and 3' -5' exoribonuclease activities, making it a potential drug target for therapeutic intervention. 2019-nCoV NSP14 shares 98.7% sequence similarity with the corresponding protein in SARS coronavirus, enabling construction of homology models for virtual screening. Based on docking scores, 18 drugs were selected for further evaluation, among which four compounds (Saquinavir, Hypericin, Baicalein, and Bromocriptine) could bind to both the N-terminal and C-terminal domains of 2019-nCoV NSP14. Considering the previously reported antiviral functions of these four drugs, we propose that their anti-2019-nCoV effects should be evaluated in 2019-nCoV infection cell culture models.

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Figure and Table Legends

Figure 1 [Figure 1: see original paper]. The binding model of Saquinavir against 2019-nCoV NSP14. (A) Interactions between Saquinavir (cyan) and associated residues (off-white) in the N-terminus of the homology model for 2019-nCoV; (B) Binding models of Saquinavir (cyan) in the 2019-nCoV NSP14 protein N-terminus pocket (white surface); (C) Interactions between Saquinavir (cyan) and associated residues (off-white) in the C-terminus of the homology

model for 2019-nCoV; (D) Binding models of Saquinavir (cyan) in the 2019-nCoV NSP14 protein C-terminus pocket (white surface). Numbers accompanying dashed yellow lines represent the interaction distance (Å).

Figure 2 [Figure 2: see original paper]. The binding model of Hypericin against 2019-nCoV NSP14. (A) Interactions between Hypericin (cyan) and associated residues (off-white) in the N-terminus of the homology model for 2019-nCoV; (B) Binding models of Hypericin (cyan) in the 2019-nCoV NSP14 protein N-terminus pocket (white surface); (C) Interactions between Hypericin (cyan) and associated residues (off-white) in the C-terminus of the homology model for 2019-nCoV; (D) Binding models of Hypericin (cyan) in the 2019-nCoV NSP14 protein C-terminus pocket (white surface). Numbers accompanying dashed yellow lines represent the interaction distance (Å).

Figure 3 [Figure 3: see original paper]. The binding model of Baicalein against 2019-nCoV NSP14. (A) Interactions between Baicalein (cyan) and associated residues (off-white) in the N-terminus of the homology model for 2019-nCoV; (B) Binding models of Baicalein (cyan) in the 2019-nCoV NSP14 protein N-terminus pocket (white surface); (C) Interactions between Baicalein (cyan) and associated residues (off-white) in the C-terminus of the homology model for 2019-nCoV; (D) Binding models of Baicalein (cyan) in the 2019-nCoV NSP14 protein C-terminus pocket (white surface). Numbers accompanying dashed yellow lines represent the interaction distance (Å).

Figure 4 [Figure 4: see original paper]. The binding model of Bromocriptine against 2019-nCoV NSP14. (A) Interactions between Bromocriptine (cyan) and associated residues (off-white) in the N-terminus of the homology model for 2019-nCoV; (B) Binding models of Bromocriptine (cyan) in the 2019-nCoV NSP14 protein N-terminus pocket (white surface); (C) Interactions between Bromocriptine (cyan) and associated residues (off-white) in the C-terminus of the homology model for 2019-nCoV; (D) Binding models of Bromocriptine (cyan) in the 2019-nCoV NSP14 protein C-terminus pocket (white surface). Numbers accompanying dashed yellow lines represent the interaction distance (Å).

Table 1 . Ten drugs selected from the N-terminal domain of the homology model.

Drug name	ZINC ID	Affinity (kcal/mol)	Status
Hypericin	ZINC000003780340	-9.7	Investigational-only
Bromocriptine	ZINC000053683151	-9.4	FDA-approved
Tanespimycin	ZINC000100014666	-9.2	Investigational-only
Idarubicin	ZINC000003920266	-9.0	FDA-approved
Emend	ZINC000027428713	-8.9	FDA-approved
Baicalein	ZINC000034114798	-8.8	World-not-FDA
Saquinavir	ZINC000029416466	-8.7	FDA-approved
Delavirdine	ZINC000018516586	-8.7	FDA-approved

Drug name	ZINC ID	Affinity (kcal/mol)	Status
Silibinin	ZINC000001530850	-8.6	Investigational-only
Golvatinib	ZINC000043195317	-8.6	Investigational-only

Table 2 . Eight drugs selected from the C-terminal domain of the homology model.

Drug name	ZINC ID	Affinity (kcal/mol)	Status
Hypericin	ZINC000003780340	-9.7	Investigational-only
Olysio	ZINC000164760756	-9.4	FDA-approved
Sovaprevir	ZINC000085537149	-9.3	Investigational-only
Celsentri	ZINC000003817234	-9.2	FDA-approved
Saquinavir	ZINC000003914596	-9.0	FDA-approved
Maraviroc	ZINC000101160855	-8.8	World-not-FDA
Baicalein	ZINC000034114798	-8.8	World-not-FDA
Bromocriptine	ZINC000053683151	-8.7	FDA-approved

Table 3 . The calculated binding energies of ligands to the N-terminus of 2019-nCoV NSP14.

Energy (kcal/mol)	Saquinavir	Hypericin	Baicalein	Bromocriptine
ΔE_{vdw}	-52.9602 ± 2.9999	-36.4737 ± 4.0922	-36.8721 ± 3.4155	-45.8461 ± 3.1764
ΔE_{ele}	-128.6886 ± 21.2732	-78.5578 ± 10.7496	-47.8615 ± 12.4900	-119.9028 ± 8.3707
ΔG_{gb}	151.4060 ± 21.5835	90.4227 ± 7.8130	66.0255 ± 11.0420	137.397 ± 6.4239
ΔG_{np}	-7.0283 ± 0.3128	-5.5659 ± 0.2085	-5.1872 ± 0.2001	-5.7839 ± 0.2553
ΔG_{cal}	-37.2711 ± 3.2160	-30.1746 ± 3.1914	-23.8953 ± 4.4800	-34.1350 ± 4.3683

*ΔE_{vdw} = van der Waals energy terms; ΔE_{ele} = electrostatic energy; ΔG_{gb} = polar solvation free energy; ΔG_{np} = nonpolar solvation free energy; ΔG_{cal} = final estimated binding free energy calculated from the above terms (kcal/mol).

Table 4 . The calculated binding energies of ligands to the C-terminus of 2019-nCoV NSP14.

Energy (kcal/mol)	Saquinavir	Hypericin	Baicalein	Bromocriptine
ΔE_{vdw}	-70.4383 ± 4.1035	-45.729 ± 2.4822	-48.6473 ± 3.5522	-61.4659 ± 2.9431
ΔE_{ele}	-38.8487 ± 7.5603	-58.4555 ± 12.1238	-192.8463 ± 18.1708	-62.3583 ± 7.0875
ΔG_{gb}	57.7780 ± 6.3018	78.5444 ± 10.3832	202.1598 ± 16.7035	70.6739 ± 5.6693
ΔG_{np}	-8.7666 ± 0.3476	-5.3546 ± 0.2317	-6.9761 ± 0.1614	-6.6602 ± 0.2480
ΔG_{cal}	-60.2757 ± 4.7708	-30.9955 ± 2.9975	-46.3099 ± 3.5689	-59.8104 ± 3.5389

Note: Figure translations are in progress. See original paper for figures.