Journal of Genetics and Genomics xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Genetics and Genomics

Journal homepage: www.journals.elsevier.com/journal-of-genetics-and-genomics/

Letter to the editor

Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines

Coronaviruses, members of the family *Coronaviridae* and subfamily *Coronavirinae*, are enveloped positive-strand RNA viruses which have spikes of glycoproteins projecting from their viral envelopes, thus exhibit a corona or halo-like appearance (Masters and Perlman, 2013; Cui et al., 2019). Coronaviruses are the causal pathogens for a wide spectrum of respiratory and gastrointestinal diseases in both wild and domestic animals, including birds, pigs, rodents, etc (Dhama et al., 2014). Previous studies have found that six strains of coronaviruses are capable to infect humans, including four strains circulating yearly to cause common cold, and other two strains which are the source for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS-CoV), respectively (Dhama et al., 2014; Cui et al., 2019).

Starting from December 2019, a novel coronavirus, which was later named 2019-nCoV ('n' stands for novel), was found to cause Severe Acute Respiratory (SARI) symptoms, including fever, dyspnea, asthenia and pneumonia among people in Wuhan, China (Zhu et al., 2020; Lu et al., 2020; Hui et al., 2020). The first batch of patients infected by 2019-nCoV were almost all connected to a seafood market in Wuhan, which also trades wild animals. Later, contact transmission of 2019-nCoV among humans was confirmed, and the number of infected patients increased rapidly in Wuhan as well as other major cities in China. A series of actions have been taken by the Chinese government to control the epidemic of the virus, and effective medical methods are in urgent needs to prevent 2019-nCoV infection and cure the disease.

Among all known RNA viruses, coronaviruses have the largest genomes, ranging from 26 kb to 32 kb in length (Regenmortel et al., 2000, Schoeman and Fielding, 2019). Besides encoding structural proteins, majority part of the coronavirus genome is transcribed and translated into a polypeptide, which encodes proteins essential for viral replication and gene expression (Lai and Holmes, 2001). The ~306 aa long main protease (M^{pro}), a key enzyme for coronavirus replication, is also encoded by the polypeptide and responsible for processing the polypeptide into functional proteins (Lai and Holmes, 2001). The M^{pro} has similar cleavage-site specificity to that of picornavirus 3C protease ($3C^{pro}$), thus is also known as 3C-like protease ($3CL^{pro}$) (Gorbalenya et al., 1989). Studies have shown that the M^{pro}s of different coronaviruses are highly conserved in terms of both sequences and 3D structures (Xue et al., 2008). These features, together with its functional importance, have made M^{pro} an attractive target for the design of anticoronaviral drugs (Anand et al., 2003; Xue et al., 2008).

To present, these are still no clinically approved antibodies or drugs specific for coronaviruses, which makes it difficult for curing 2019-nCoV caused diseases and controlling the associated pandemic. With the hope to identify candidate drugs for 2019-nCoV, we adopted a computational approach to screen for available commercial medicines which may function as inhibitors for the M^{pro} of 2019-nCoV.

A previous attempt to predict drugs for the M^{pro} of SARS-CoV has identified two HIV-1 protease inhibitors, namely lopinavir and ritonavir, as potential candidates, both of which bind to the same target site of M^{pro} (Nukoolkarn et al., 2008). Clinical application of these two drugs on 2019-nCoV patients also appears to be effective, demonstrating the importance of the drug binding site for suppressing 2019-nCoV M^{pro} activity.

To search for other drugs that may inhibit 2019-nCoV M^{pro}, we first evaluated the sequence and structural conservation of lopinavir/ritonavir binding site between SARS-CoV and 2019-nCoV. The protein sequences of SARS-CoV M^{pro} and 2019-nCoV M^{pro} are 96% identical (Fig. 1A), and the spatial structure of the previously reported lopinavir/ritonavir binding pocket is also conserved between SARS-CoV M^{pro} and 2019-nCoV M^{pro} (Fig. 1B). During the submission process of this manuscript, the crystal structure of 2019-nCoV M^{pro} was solved (PDB ID: 6LU7), of which the lopinavir/ritonavir binding pocket region is nearly identical to our predicted model (Fig. S1). The conserved amino acids Thr24-Asn28 and Asn119 (numbered according to positions in SARS-CoV M^{pro} because the present sequence of 2019-nCoV M^{pro} in GenBank is in polypeptide form) formed the binding pockets for lopinavir/ritonavir in the spatial structure of both SARS-CoV M^{pro} and 2019-nCoV M^{pro}, whereas the nearby non-conserved amino acids locate far away from the binding pocket, thus would not affect its structural conservation (Fig. 1B). Virtual docking of lopinavir/ritonavir to 2019-nCoV M^{pro} also showed high binding ability to the pocket site (Fig. 1C), similar to previous report for SARS-CoV M^{pro} (Nukoolkarn et al., 2008). Amino acids Thr24, Thr26, and Asn119 were predicted to be the key residues for binding to the drugs (Figs. 1C and S2), forming 2 hydrogen bonds with lopinavir and 3 hydrogen bonds with ritonavir, respectively.

Based on these results, we performed virtual docking to screen for commercial medicines in the DrugBank database that could bind to the above mentioned pocket site of 2019-nCoV M^{pro}, and identified 10 candidate clinical medicines (Table 1; Figs. 1B and S3). These drugs could form hydrogen bonds with one or more residues among Thr24-Asn28 and Asn119, therefore theoretically, are capable to bind to the pocket formed by these amino acids and interfere the function of 2019-nCoV M^{pro}.

In summary, based on the structural information of clinical effective medicines for 2019-nCoV, we have predicted a list of

https://doi.org/10.1016/j.jgg.2020.02.001

Please cite this article as: Liu, X., Wang, X.-J., Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines, Journal of Genetics and Genomics, https://doi.org/10.1016/j.jgg.2020.02.001

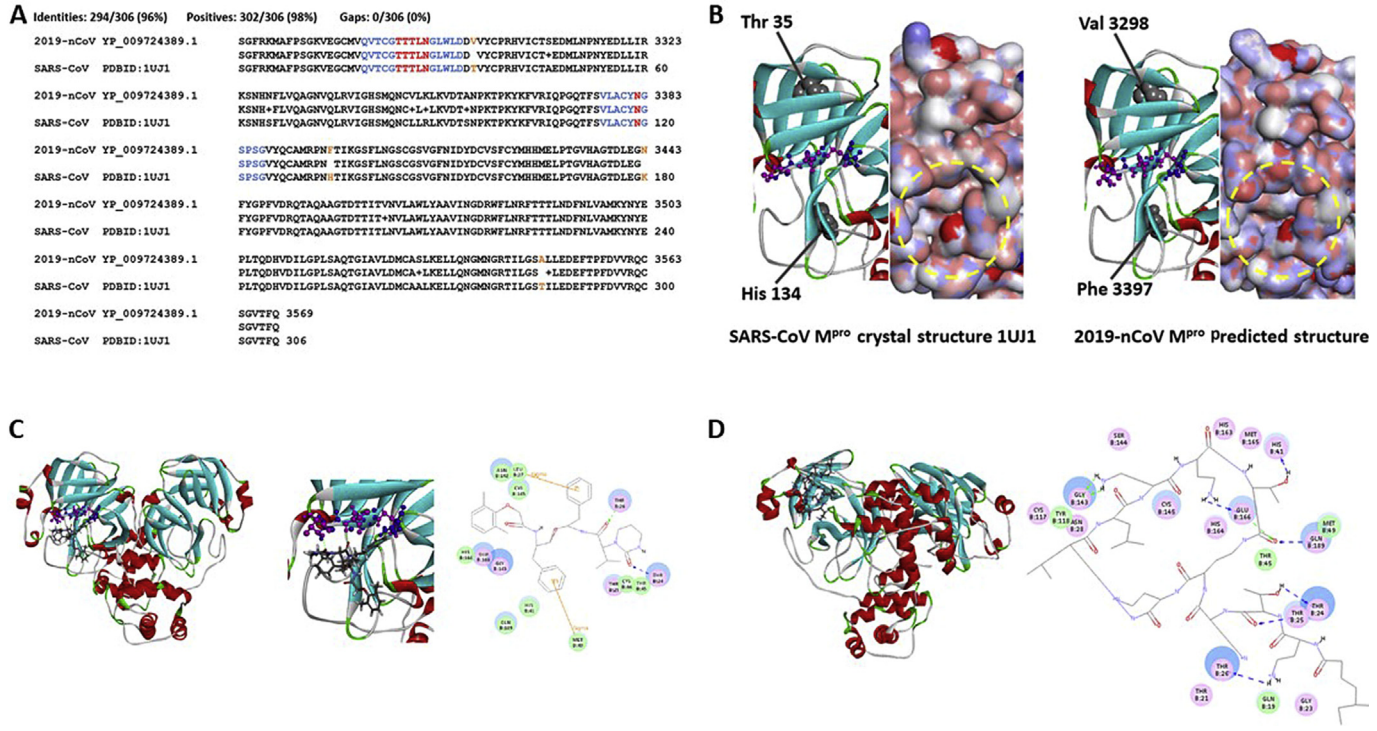

Fig. 1. Screen for potential 2019-nCoV M^{pro} inhibitors from commercial medicines. **A**: Sequence comparison between 2019-nCoV M^{pro} and SARS-Cov M^{pro}. Amino acids forming hydrogen bonds with drugs are shown in red, and their adjacent 5 amino acids on each side are shown in blue. Mutated amino acids rather than positive substitutions are shown in orange. **B**: Structural comparison of lopinavir/ritonavir binding pocket in SARS-CoV M^{pro} and 2019-nCoV M^{pro}. Ribbon models show the pocket structure of SARS-CoV M^{pro} (left) and 2019-nCoV M^{pro} (right), with α-helixes shown in red and β-sheets in cyan. Residues essential for lopinavir/ritonavir binding are shown in ball-and-stick format, of which Thr24, Thr26, and Asn28 are shown in purple, and Thr25, Leu27, and Asn119 are shown in blue. Protein solid surface model is shown to the right of each ribbon model, with the outer rim of the binding pocket marked by dashed yellow cycle. Mutations (marked in orange in panel **A**) are shown as gray balls, which are apart from the binding pocket. **C**: Docking model of lopinavir to 2019-nCoV M^{pro}. Left, overall docking model of lopinavir to 2019-nCoV M^{pro}; middle, enlargement of the lopinavir binding region; right, predicted chemical bonds between lopinavir and key residues of the binding pocket. **D**: Docking model of colistin to 2019-nCoV M^{pro}. Left, overall docking model of colistin to 2019-nCoV M^{pro}; right, predicted chemical bonds between colistin and key residues of the binding pocket. In (**C**) and (**D**), protein ribbon models are shown with the same diagram as described in (**B**), and drugs are shown as sticks. Hydrogen bonds between drugs and amino acids are shown as dash lines, and Pi bonds are shown as orange lines.

Table 1
Predicted commercial medicines as potential inhibitors against 2019-nCoV M^{pro}.

Name	H-bond count	Vital residues for H-bond formation	Medical indications in DrugBank
Colistin	9	THR24, THR25, THR26	Antibiotic
Valrubicin	7	THR24, THR25, THR26, ASN28, ASN119	Anthracycline, antitumor
Icatibant	6	ASN28, ASN119	Hereditary angioedema
Bepotastine	5	THR25, THR26, ASN119	Rhinitis, uriticaria/ puritus
Epirubicin	4	ASN28, ASN119	Antitumor
Epoprostenol	4	ASN119	Vasodilator, platelet aggregation
Vapreotide	3	THR24, ASN28, ASN119	Antitumor
Aprepitant	3	ASN28, ASN119	Nausea, vomiting, antitumor
Caspofungin	3	ASN119	Antifungal
Perphenazine	2	ASN28, ASN119	Antipsychotic

commercial medicines which may function as inhibitors for 2019-nCoV by targeting its main protease M^{pro}. Compared to lopinavir/ritonavir, most of these predicted drugs could form more hydrogen bonds with 2019-nCoV M^{pro}, thus may have higher mutation tolerance than lopinavir/ritonavir. It should be noted that these results were obtained solely by *in silico* predictions, further experiments are needed to validate the efficacy of these drugs. The binding pockets of these drugs on M^{pro} are conserved between SARS-CoV M^{pro} and 2019-nCoV M^{pro}, indicating the potential of these drugs as inhibitors for other coronaviruses with similar M^{pro} binding sites and pocket structures.

Acknowledgements

This work is supported by grants from CAS Advance Research Programs (QYZDJ-SSW-SMC015) and National Natural Science Foundation of China (91940304 and 81790622) to X.-J. W.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgg.2020.02.001.

References

Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J.R., Hilgenfeld, R., 2003. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science 300, 1763–1767.
Cui, J., Li, F., Shi, Z.L., 2019. Origin and evolution of pathogenic coronaviruses. Nat. Rev. Microbiol. 17, 181–192.
Dhama, K., Pawaiya, R., Chakraborty, S., Tiwari, R., Saminathan, M., Verma, A., 2014. Coronavirus infection in equines: a review. Asian J. Anim. Vet. Adv. 9, 164–176.
Gorbalenya, A.E., Donchenko, A.P., Blinov, V.M., Koonin, E.V., 1989. Cysteine proteases of positive strand RNA viruses and chymotrypsin-like serine proteases. A distinct protein superfamily with a common structural fold. FEBS Lett. 243, 103–114.
Hui, D.S., E, I.A., Madani, T.A., Ntoumi, F., Kock, R., Dar, O., Ippolito, G., McHugh, T.D., Memish, Z.A., Drosten, C., Zumla, A., Petersen, E., 2020. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - the latest 2019 novel coronavirus outbreak in Wuhan, China. Int. J. Infect. Dis. 91, 264–266.
Lai, M.M.C., Holmes, K.V., 2001. Coronaviridae: the viruses and their replication. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology. Lippincott Williams & Wilkins, Philadelphia, pp. 1163–1179.
Lu, H., Stratton, C.W., Tang, Y.W., 2020. Outbreak of pneumonia of unknown etiology

Please cite this article as: Liu, X., Wang, X.-J., Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines, Journal of Genetics and Genomics, https://doi.org/10.1016/j.jgg.2020.02.001

in Wuhan China: the mystery and the miracle. J. Med. Virol. https://doi.org/10.1002/jmv.25678.
Masters, P.S., Perlman, S., 2013. Coronaviridae. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology. Lippincott Williams & Wilkins, Philadelphia, pp. 825–858.
Nukoolkarn, V., Lee, V.S., Malaisree, M., Aruksakulwong, O., Hannongbua, S., 2008. Molecular dynamic simulations analysis of ritonavir and lopinavir as SARS-CoV 3CL(pro) inhibitors. J. Theor. Biol. 254, 861–867.
van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., Wickner, R.B., 2000. Coronaviridae. In: van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., et al. (Eds.), Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, pp. 835–849.
Schoeman, D., Fielding, B.C., 2019. Coronavirus envelope protein: current knowledge. Virol. J. 16, 69.
Xue, X., Yu, H., Yang, H., Xue, F., Wu, Z., Shen, W., Li, J., Zhou, Z., Ding, Y., Zhao, Q., Zhang, X.C., Liao, M., Bartlam, M., Rao, Z., 2008. Structures of two coronavirus main proteases: implications for substrate binding and antiviral drug design. J. Virol. 82, 2515–2527.
Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F., Tan, W., 2020. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. https://doi.org/10.1056/NEJMoa2001017.

Xin Liu
Key Laboratory of Genetic Network Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Xiu-Jie Wang*
Key Laboratory of Genetic Network Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

University of Chinese Academy of Sciences, Beijing 100049, China

Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing 100101, China

* Corresponding author.
E-mail address: xjwang@genetics.ac.cn (X.-J. Wang).

28 January 2020
Available online xxx

Please cite this article as: Liu, X., Wang, X.-J., Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines, Journal of Genetics and Genomics, https://doi.org/10.1016/j.jgg.2020.02.001