Furin, a potential therapeutic target for COVID-19

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2020-02-23

ChinaRxiv

Abstract

A novel coronavirus (SARS-CoV-2) infectious disease outbreak emerged in Wuhan, Hubei Province in December 2019, spreading rapidly to other regions and was subsequently declared a Public Health Emergency of International Concern. We compared the spike proteins from four sources—SARS-CoV-2, SARS-CoV, MERS-CoV, and Bat-CoV RaTG13—and identified redundant PRRA sequences in the SARS-CoV-2 viral sequence. Through a series of analyses, we propose the mechanism underlying the higher infectivity of SARS-CoV-2 compared to other coronaviruses. Furthermore, through structure-based virtual ligand screening, we identified potential furin inhibitors that may have therapeutic applications for COVID-19.

Full Text

Furin, a potential therapeutic target for COVID-19

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Abstract A novel coronavirus (SARS-CoV-2) infectious disease has broken out in Wuhan, Hubei Province since

December 2019, and spread rapidly from Wuhan to other areas, which has been listed as an international

concerning public health emergency. We compared the Spike proteins from four sources, SARS-CoV-2,

SARS-CoV, MERS-CoV and Bat-CoVRaTG13, and found that the SARS-CoV-2 virus sequence had redundant

PRRA sequences. Through a series of analyses, we propose the reason why SARS-CoV-2is more infectious than

other coronaviruses. And through structure based virtual ligand screening, we foundpotentialfurin inhibitors, which

might be used in the treatment of new coronary pneumonia.

Keywords:SARS-CoV-2; Spike proteins; Furin; Inhibitors; Virtual screening

1. Introduction In December 2019, a series of acute respiratory diseases occurred in Wuhan, Hubei Province,

China and then spread rapidly from Wuhan to other areas. As of February 17, 2020, a total of

71,444 patients have been diagnosed and 1,775 have died worldwide. This is caused by a novel

coronavirus, which was named as "2019-nCoV" by the World Health Organization, and diseases

caused by 2019-nCoV was COVID-19. 2019-nCoV, as a close relative of SARS-CoV, was

classified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International

Committee on Taxonomy of Viruses (ICTV) on February 11, 2020.

Coronaviruses (CoVs) are mainly composed of four structural proteins, including Spike (S),

membrane (M), envelope (E) and nucleocapsid (N) [1]. Spike, a trimeric glycoprotein of CoVs,

determines diversity of CoVs and host tropism, and mediates CoVs binding to host cells

surface-specific receptors and virus-cell membrane fusion [2]. Current research found that

SARS-CoV-2 belongs to the beta coronavirus genus, and speculated that it may interact with

angiotensin-converting enzyme 2 (ACE2) on the surface of human cells through Spike protein,

thereby infecting human respiratory epithelium cell [3]. Letko M and Munster Vthen identified the

receptor for SARS-CoV-2 entry into human cells to be ACE2 [4].

Coronavirus Spike protein plays a key role in the early stages of viral infection, with the S1

domain responsible for receptor binding and the S2 domain mediating membrane fusion [5]. The

process of SARS-CoV infecting the host involves two indispensable cleaving processes which

affect the infectious capacity of SARS-CoV. First, Spike was cleaved into receptor-bound

N-terminal S1 subunit and membrane-fusion C-terminal S2 subunit by host proteases at S1/S2

cleavage site (such as type II transmembrane serine protease (TMPRSS2), cathepsins B and L) [6,7].

Second, after CoVs are endocytosed by the host, the lysosomal protease mediates cleavage of S2

subunit (S2' cleavage site) and releases the hydrophobic fusion peptide to fuse with the host cell

membrane [8].

Furin, a kind of proprotein convertases (PCs), is located in the trans-Golgi network (TGN) and

activated by acid pH [9]. Furin can cleave precursor proteins with specific motifs to produce

mature proteins with biological activity. The first (P1) and fourth (P4) amino acids at the

N-terminus of the substrate cleavage site must be arginine "Arg-X-X-Arg ↓" (R-X-X-R, X: any

amino acid, ↓:cleavage site). If the P2 position is basic lysine or arginine, the cleavage efficiency

can be improved by about 10 times [10]. Kibler KV et al. demonstrated that the Spike protein S1/S2

and S2′ cleavage sites of the infectious bronchitis virus (IBVs) Beaudette strain can be recognized

by fruin, which is a distinctive feature of IBV-Beaudette with other IBVs and has stronger

infection ability [11,12]. Based on the characteristics of furin' s recognition substrate sequence, some

short peptide inhibitors have been developed, such as

Decanoyl-Arg-Val-Lys-Arg-chloromethylketone (Dec-RVKR-CMK) and modified ( )1-antitrypsin

Portland (( )1-PDX). However, the non-specific and irreversible inhibitory effects on all members

of the PC family limit their application [10, 13]. No small molecule inhibitor of furin with good

effect and high specificity has been found so far.

The epidemiological observations showed the infectious capacity of SARS-CoV-2 is stronger

than SARS-CoV, so there are likely to be other mechanisms to make the infection of SARS-CoV-2

easier. We suppose the main possibilities as follows, first, SARS-CoV-2 RBD combining with

ACE2 may have other conformations; second, the SARS-CoV-2 Spike protein can also bind to

other receptors besides ACE2; third, Spike is more easily cleaved by host enzymes and easily

fuses with host cell membrane. We compared the Spike proteins from four sources, SARS-CoV-2,

SARS-CoV, MERS-CoV and Bat-CoVRaTG13, and found that the SARS-CoV-2 virus sequence

had redundant PRRA sequences. Through a series of analyses, this study propose that one of the

important reasons for the high infectivity of SARS-CoV-2 is a redundant furin cut site in its Spike

protein.And through structure based virtual ligand screening, we proposed possible furin inhibitors,

which might be potentially used in the treatment of COVID-19.

2. Methodology 2.1 Homology Spike protein blast and sequence alignment.

The Spike protein of(GB:QHR63250.1) was downloaded from NCBI nucleotide database.

The protein sequence were aligned with whole database using BLASTp to search for homology

viral Spike protein (Alogorithm parameters, Max target sequences: 1000, Expect threshold: 10).

Multiple-sequence alignment was conducted in BLASTp online and analysis with DNAMAN and

Jalview. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 7

software package. The percentage of replicate trees in which the associated taxa clustered together

in the bootstrap test wasdetermined by 500 replicates. The Spike protein sequence analyses were

conducted in snapgene view.

2.2 Furin cleavage site prediction

The prediction of furin cleavage sites were carried out in ProP 1.0 Server

(http://www.cbs.dtu.dk/services/ProP/).

2.3 Compounds database

Approved drug database was from the subset of ZINC database, ZDD (ZINC drug database)

containing 2924 compounds [14]. Natural products database was constructed by ourselves,

containing 1066 chemicals separated from traditional Chinese herbals in own lab and

natural-occurring potential antiviral components and derivatives. Antiviral compounds library

contains 78 known antiviral drugs and reported antiviral compounds through literature search.

2.4 Homology modeling and molecular docking

Corresponding homology models predicted by Fold and Function Assignment System server

for each target protein were downloaded from Protein Data Bank (www.rcsb.org). Alignment of

two protein sequences and subsequent homology modeling were performed by bioinformatics

module of ICM 3.7.3 modeling software on an Intel i7 4960 processor (MolSoft LLC, San Diego,

CA). For the structure-based virtual screening, ligands were continuously resiliently made to dock

with the targetthat was represented in potential energy maps by ICM 3.7.3 software, to identify

possible drug candidates. 3D compounds of each database were scored according to the internal

coordinate mechanics (Internal Coordinate Mechanics, ICM)[15]. Based on Monte Carlo method,

stochastic global optimization procedure and pseudo-Brownian positional/torsional steps, the

position of intrinsic molecular was optimized. By visually inspecting, compounds outside the

active site, as well as those weakly fitting to the active site were eliminated. Compounds with

Scores less than -30 or mfScores less than -100 (generally represents strong interactions) have

priority to be selected. Protein-protein docking procedure was performed according to the

ICM-Pro manual.

3. Results 3.1 Bioinformatics analysis reveals furin cut site in Spike protein of SARS-CoV-2

By sequence alignment of Spike protein sequence of SARS-CoV-2 with its highly

homologous sequences, it was found that cleavage site Spike of SARS-CoV-2 had 4 redundant

amino acids-PRRA, and these were not found in those of high homology coronavirus, which

formed a furin-like restriction site as RRAR(Figure S1). Through prediction in ProP 1.0 Server, it

was found the sequence was indeed easily digested by furin(Figure S2). In order to explore the

evolution of this sequence, we used the BLASTp method to find 1,000 homologous Spike

sequences with homology from 100% to 31%, which all from beta CoVs. Multiple sequence

alignments were performed on these thousands of Spike sequences. One sequence was selected

from each highly homologous class (homology greater than 98.5%) for further sequence

alignment, and about 155 sequences were finally selected. A homologous multiple sequence

alignment was performed on these 155 sequences, and then a phylogenetic tree was

constructed(Figure 1). It is found from the phylogenetic tree that the Spike of SARS-CoV-2

exhibited the closest linkage to those of Bat-SL-CoV and SARS-CoV, and far from those of

MERS-CoV, HCoV-HKU1, HCoV-OC43. In general, most of the Spike protein in α-CoV does not

have a furin cleavage site, most of that in gama-CoV has a furin cleavage site, and that in

beta-CoV with or without furin cleavage site are common[16].

We performed furin digestion site prediction on the sequence of each type of coronavirus

Spikethrough online software. It was found that all Spike with a SARS-CoV-2 Spike sequence

homology greater than 40% did not have a furin cleavage site (Figure 1, Table 1), including

Bat-CoV RaTG13 and SARS-CoV (with sequence identity as 97.4% and 78.6%, respectively).

The furin cleavage site "RRAR"in SARS-CoV-2 is unique in its family, rendering by its unique

insert of "PRRA" . The furin cleavage site of SARS-CoV-2 is unlikely to have evolved from

MERS, HCoV-HKU1, and so on. From the currently available sequences in databases, it is

difficult for us to find the source. Perhaps there are still many evolutionary intermediate sequences

waiting to be discovered.

By analysis of the SARS -CoV-2 Spike protein sequence, it was found that most features are

similar to SARS-CoV. It has an N-terminal signal peptide and is divided into two parts, S1 and S2.

Among them, S1 contains N-terminal domain and receptor binding region. And S2 is mainly

responsible for membrane fusion. The C-terminal region of S2 is S2' , containing a fusion peptide,

Hetad repeat1, Hetad repeat 2, and a transmembrane domain(Figure 2). There are two cleavage

sites between S1 and S2 ' , named CS1 and CS2. However, there are some differences in this two

cleavage sites.

Unlike SARS-CoV, SARS-CoV-2 contains polybasic amino acids (RRAR) at the CS1

digestion site, and trypsin digestion efficiency will be significantly improved here[5]. More

importantly, as mentioned above, this site can be recognized and cleaved by the furin enzyme. The

cleavage of Spike protein promotes structural rearrangements of RBD for the adaptation to

receptor, thus increasing the affinity[17]. More importantly, the digestion of Spike is an

indispensable for membrane fusion of S2 part[18]. In this case, the efficiency of the

SARS-CoV-2Spike protein cleavage is significantly higher than that of SARS-CoV, and the

SARS-CoV-2Spike protein could be cut during the process of virus maturation (Figure 3). The

receptor affinity and membrane fusion efficiency of SARS-CoV-2 would be significantly

enhanced compared to that of SARS-CoV. The membrane fusion of SARS-CoV-2Spike protein is

more likely to occur during endocytosis process. This may explains the current strong infectious

capacity of SARS-CoV-2. So, the development of furin inhibitors may be a promising approach to

block its transmissibility.

Figure1.Evolutionary relationships of taxa.The evolutionary history was inferred using the Neighbor-Joining method. The

bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches

corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were

computed using the Poisson correction method and in the units of the number of amino acid substitutions per site. The analysis

involved 155 amino acid sequences. All positions containing gaps and missing data were eliminated. There are a total of 711

positions in the final dataset. Evolutionary analyses were conducted in MEGA7. Those painted in red mean containing cleavage

site in sequences and those painted in yellow mean no cleavage site in sequences.

Figure 2.Sequence analysis of Spike protein in SARS-CoV-2. It contains an N-terminal signal peptide, S1 and S2. S1 contains

N-terminal domain and receptor binding region. And S2 is mainly responsible for membrane fusion. The C-terminal region of S2

is S2' , it contains a fusion peptide, HR1, HR2, and a transmembrane domain, the amino acid sequence numbers of every domain

are annotated below them. Cleavage sites contained in SARS-CoV and SARS-CoV-2 are marked by rhombus.

Figure 3.A schematic diagram of the process of SARS-CoV and SARS-CoV-2 infecting host cells.Those protease are

presented by sector in different colors. Furin can cleaveSpike in the process of viral maturation.

Table 1.Furin cleavage probability of Spike sequence homology

Description Accession no. CS1 sequence Furinscorea Identityb

SARS-CoV-2 QHR63250.1 NSPRRAR/SV 0.620 100%

Bat-CoV-RaTG13 QHR63300.1 QTQTNSR/SV 0.151 97.4%

Bat-SL-CoV AVP78042.1 HTASILR/ST 0.170 80.3%

SARS-CoV ABF68955.1 QLTPAWR/IY 0.117 76.0%

Bat-CoV HKU5 AGP04941.1 PSARLAR/SD 0.697 37.1%

MERS-CoV QBM11737.1 LTPRSVR/SV 0.563 35.0%

Rat-CoV AFG25760.1 TAHRARR/SV 0.879 36.3%

MHV ABS87264.1 TSHRARR/SI 0.861 36.9%

HCoV-HKU1 AGT17758.1 SSRRKRR/GI 0.744 36.8%

Rodent-CoV ATP66727.1 TARRKRR/AL 0.795 37.3%

Beta-CoVsp AYR18670.1 ATRRAKR/DL 0.753 35.9%

Equine-CoV BAS18866.1 TARRQRR/SP 0.815 37.1%

Porcine-CoV ARC95227.1 TSLRSRR/SL 0.758 36.1%

Bovine-CoV QGW57589.1 TKRRSRR/AI 0.780 37.5%

Canine-CoV ABG78748.1 TQRRSRR/SI 0.832 37.1%

Camel-CoV HKU23 ALA50080.1 IDRRARR/FT 0.718 36.5%

Rabbit-CoV HKU14 AFE48805.1 TLQPSRR/AI 0.629 37.7%

Human-CoV OC43 AMK59677.1 KTRRSRR/AI 0.720 36.8%

a Scores are predicted by ProP 1.0 Server. Scores above 0.5 mean furin cleavable.

b Identities compared with SARS-CoV-2 Spike protein.

3.2 Homology modeling and protein-protein docking calculation

In our previous studies (accepted by ActaPharmaceuticaSinica B), both SARS and

SARS-CoV-2 spike RBD structures have been docked with human ACE2 to calculate their

binding free energy. In that time, the complex structure of SARS-CoV-2 RBD with ACE2 was not

available.Its energy was calculated based on the homology model generated from

SARS_RBD-ACE2 complex. The binding energy between the SARS-CoV-2 spike RBD and

human ACE2 was -33.72 kJ mol-1, and that between SARS-CoV spike RBD and ACE2 was -49.22

KJ mol-1.This means the binding affinity between SARS-CoV-2 spike and ACE2 is weaker than

that of SARS spike. During this manuscript was prepared, the structure of SARS-CoV-2 spike

RBD-ACE2 complex was disclosed[19]. Based on this new real structure of SARS-CoV-2 spike

RBD-ACE2 complex, we re-did the calculation and found that the binding free energy between

SARS-CoV-2 spike RBD and ACE2 was -50.13 KJ mol-1 (Figure S3). This means the binding

affinity between SARS-CoV-2 spike and ACE2 is slightly stronger than that of SARS spike.By

inspecting the crystal structure of SARS-CoV-2 RBD-ACE2 complex and SARS RBD-ACE2

complex, one can find that one key loop of SARS-CoV-2 RBD in the complex interface had very

different conformation compared to that of SARS RBD and previous modeled SARS-CoV-2 RBD

(Figure S4).

In order to further explore the possible mechanism how furin cleaves SARS-CoV-2 Spike,

we perform protein-protein docking for furin and Spike. Although a Cryo-EM structure of

SARS-CoV-2 Spike has been published in bioRxiv during this manuscript was prepared[20], the

PDB coordinate was still not available so far. We already built a homology model of

SARS-CoV-2 Spike in our previous paper submitted to another regular journal. SARS-CoV-2

Spike structure was built by using the SARS-CoVSpike structure as the temple (PDB code:

5X58)[21]. By superimposing the SARS-CoVSpike with the SARS-CoV-2 Spike, we can find that

the major conformation differences between two structures are RBD domain, Arg685/677 loop

region(furin/trypsin/TMPRSS2 cut site) and S2 loop region just after fusion peptide (Figure

4A).The trypsin/TMPRSS2 cut site of SARS-CoV was disordered and missing from the original

Cryo-EM structure possibly due to its flexibility and without electro density. The "PRRA"

inserting in SARS-CoV-2 in this region apparently generate the more flexible loop region and

accessible cut site for protease. We performed protein-protein docking by setting SARS-CoV-2

Spikefurincleavage loop as the receptor, and furin active pocket as the ligand. The protein-protein

docking results showed that furin acidic/negative active pocket can be well fitted onto the

SARS-CoV-2 Spikebasic/positive S1/S2 protease cleavage loop with low energy (-18.43

Kcal/mol). This implies that the extra "PRRAR" loop of SARS-CoV-2 Spike renders it more

fragile to the protease. And this may allow this site to be cut during the maturation, efficiently

enhancing the infection efficiency.

chinaXiv:202002.00062v1 Figure 4.Protein-protein docking model of SARS-CoV-2 Spike with furin. (A) Superimposition of SARS-CoVSpike and

SARS-CoV-2 Spike. Two S1/S2 protease cleavage sites and fusion peptide were shown as electrostatic surface mode. (B) Furin

was docked onto the putative furin cut site (Arg685) of SARS-CoV-2 Spike. Both domains are shown as electrostatic surface

mode.

3.3. Virtual ligand screening of furin protein

Structure-based virtual ligand screening method was used to screen potential furin protein

inhibitors through ICM 3.7.3 modeling software (MolSoft LLC, San Diego, CA) from a ZINC

Drug Database (2924 compounds), a small in-house database of natural products (including

reported common antiviral components from traditional Chinese medicine) and derivatives (1066

compounds), and an antiviral compounds library contains 78 known antiviral drugs and reported

antiviral compounds. Compounds with lower calculated binding energies (being expressed with

scores and mfscores) are considered to have higher binding affinities with the target protein.

The screening results for the ZINC Drug Database (Table 2) showed that antitumor drugs

Aminopterin, Fludarabine phosphate and Irinotecan, antibacterial drugs

Sulfoxone,Lomefloxacinand Cefoperazone,antifungaldrug Hydroxystilbamidine, antivirus

drugValganciclovir,hepatoprotective drugSilybin,folic acid supplementFolinic acid have higher

binding affinity to furin with mfscores lower than -100 or Scores lower than -30.

Here, we show one example of screen hits, Hydroxystilbamidine, which was predicted to

bind in the active site of furin with low binding energy. In the generated docking model,

Hydroxystilbamidine was well fitted into the binding pocket of the substrate and adopted similar

conformation as substrate analogous inhibitor MI-52 in PDB model 5JXH[22],occupied two arms'

position of MI-52 (Figure 5A). Asp159, Asp259 and Asp306 were predicted to form three

hydrogen bonds with imine groups of compounds (Figure 5B). It looks like that

Hydroxystilbamidine mimic at least two arginines. Weak hydrophobic interaction between His194,

Leu227, the backbone of Trp254 and Asn295 with the compound may further stabilize its

conformation.

Table 2. Potential furin inhibitors from ZINC drug database

No. Drug Name Structure Pharmacological functions

1 Aminopterin Anti-tumor

Vitamin B9, necessary

2 Folic acid material for the growth and

reproduction of body cells

3 Sulfoxone Antibacterial effect

Silybin Hepatoprotective effect

Diminazene Insecticidal effect

Fludarabine phosphate Anti-tumor

L-Arginine Nutritional supplement

Hydroxystilbamidine Antifungal effect

Antineoplastic, Methotrexate antirheumatic effects

Treatment of Parkinson' s L-dopa disease

Irinotecan Anti-tumor

Cefoperazone Antibacterial effect

Folinic acid Folic acid supplement

Intermediate for serine Glycerol 3-phosphate synthesis

Valganciclovir Antivirus

Treatment of nausea and

Fosaprepitant vomiting induced by

chemotherapy

Lomefloxacin Antibacterial effect

Glutathione Hepatoprotective effect

Treatment of 19 Famotidine gastrohelcosis

20 Imatinib Anti-tumor

21 Chenodeoxycholic acid Dissolving gallstones

Figure 5.Low-energy binding conformations of Hydroxystilbamidine bound to furin generated by molecular docking. (A)

Hydroxystilbamidinewas fitted well in the active pocket of human furin, and furin was shown as electrostatic surface model.

Hydroxystilbamidine (yellow) was overlapped with substrate analogue inhibitor MI-52 (purple).(B) Detailed view of

Hydroxystilbamidinebinding in the activepocket of furin.

Another example was anticancer drug Imatinib. It was also predicted to bind in the active site

of furin. In the generated docking model, Imatinib was fitted well in the binding pocket, and

occupied the top two arms' position of MI-52 (Figure 6A). Two hydrogen bonds were predicted to

form between the compound with Glu236 and Gly255. Weak hydrophobic interaction between

Val231, Pro256, Trp254 and Gly294 and the compound was found (Figure 6B).

Figure 6. Low-energy binding conformations of Imatinibto furin generated by molecular docking. (A) Imatinibwas fitted

well in the active pocket of human furin, and furin was shown as electrostatic surface model. Imatinib (yellow) was overlapped

with substrate analogue inhibitor MI-52 (purple).(B) Detailed view of Imatinib-binding in the activepocket of furin.

Table 3.Potential furin inhibitors from in-house natural product database

Pharmacological No. Drug Name Structure Source functions

Antioxidation,

1 (-)-Epigallocatechin gallate anti-tumor, treatment Camellia sinensis

of depression

Antioxidant effect, 2 Theaflavin 3,3' -di-O-gallate Camellia sinensis anti-tumor, anti-virus

Anti-virus Ficusbenjamina 3 Biorobin

14-deoxy-11,12- Anti-virus, Andrographispanicu

4 didehydroandrographiside anti-inflammatory lata

effect

(1S,2R,4aS,5R,8aS)-1-

formamido-1,4a-dimethyl-6- Anti-virus, Andrographolide methylene-5-((E)-2-(2-oxo-2,5- 5 anti-inflammatory derivatives dihydrofuran- 3-yl)ethenyl) effect decahydronaphthalen-2-yl

5-((R)-1,2-dithiolan-3-yl) pentanoate

2β,30β-dihydroxy-3,4-seco-friedelolact Anti-virus Viola diffusa one-27-lactone

Anti-virus Phyllanthusemblica 7 Phyllaemblicin G7

Anti-virus, Andrographispanicu

8 Andrographolide anti-inflammatory lata

effect

Anti-virus, 14-deoxy-11,12- Andrographispanicu 9 anti-inflammatory didehydroandrographolide lata effect

(1S,2R,4aS,5R,8aS)-1-

formamido-1,4a-dimethyl-6- Anti-virus, methylene-5-((E)-2-(2-oxo-2,5- Andrographolide 10 anti-inflammatory dihydrofuran-3-yl)ethenyl) derivatives effect decahydronaphthalen-2-yl

2-aminoacetate

2-[[2-O-(6-deoxy-α-L-mannopyranosyl Anti-virus,)-β-D-xylopyranosyl]oxy]-1,8-dihydro 11 anti-inflammatory Swertiakouitchensis xy-6-methoxy-9Heffect xanthen-9-one

Anti-virus,

12 Kouitchenside J anti-inflammatory Swertiakouitchensis

effect

Spatholobussuberect 13 Stigmast-5-en-3-ol Antioxidant effect usdunn

Anti-virus,

14 Kouitchenside F anti-inflammatory Swertiakouitchensis

effect

For the natural products (Table 3), a series of compounds with antivirus and

anti-inflammation effects, such as (-)-Epigallocatechin gallateand Theaflavin

3,3' -di-O-gallatefromCamellia sinensis,Biorobin from Ficusbenjamina, Andrographolide and

14-deoxy-11,12-didehydroandrographiside from Andrographispaniculata, one Andrographolide

derivative (1S,2R,4aS,5R,8aS)-1-

formamido-1,4a-dimethyl-6-methylene-5-((E)-2-(2-oxo-2,5-dihydrofuran-3-yl)ethenyl)decahydro

naphthalen-2-yl 5-((R)-1,2-dithiolan-3-yl)pentanoate, 2β,30β-dihydroxy-

3,4-seco-friedelolactone-27-lactone from Viola diffusa, Phyllaemblicin

G7fromPhyllanthusemblica, three

xanthones2-[[2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-xylopyranosyl]oxy]-1,8-dihydroxy-6-meth

oxy-9H-xanthen-9-one, Kouitchenside J and Kouitchenside Ffrom Swertiakouitchensisexhibited

high binding affinity to furin protein (mfscores< -100), suggesting the potential utility of these

compounds in the treatment of SARS-CoV-2.

(-)-Epigallocatechin gallate (EGCG) was predicted to bind in the active site of furin, as

Imatinib, it occupied the top two arms' position of MI-52 (Figure 7A). Two hydrogen bonds were

predicted formed between the compound with Asp258 and Ala292. Weak hydrophobic

interactions between Pro256, Trp254 and Gly294 and the compound were predicted (Figure 7B).

Figure 7. Low-energy binding conformations of ECCG to furin generated by molecular docking. (A) ECCGwas fitted well

in the active pocket of human furin, and furin was shown as electrostatic surface model. ECCG (yellow) was overlapped with

substrate analogue inhibitor MI-52 (purple).(B) Detailed view of ECCG binding in the activepocket of furin.

The database of 78 antiviral drugs including compounds already on the market and currently

undergoing clinical trials to treat SARS-CoV-2 infections was further screened. The results were

shown in Table 4. DNA topoisomerase II inhibitorSuramin treating hand-foot-and-mouth disease

exhibited the highest affinity with furin (mfscore = 190.406). A series HIV-1 therapeutic drugs,

such as Indinavir, Tenofoviralafenamide, TenofovirDisoproxil and Dolutegravir, and hepatitis C

therapeutic drugs, Boceprevir and Telaprevir also have high binding affinity to furin.

Suramin was predicted to bind in the active site of furin with high binding mfScores. From

generated docking model, Suramin occupied the top right arm and bottom arm positions of MI-52,

it extended more to another adjacent pocket and covered almost all the surface areas for furin

substrate binding (Figure 8A). Asp154, Asp228, Gly229, Ser253, Asp264, Glu271, Ile312, Lys449,

Arg490 and Asp530 were predicted to form 10 hydrogen bonds with the compound. Weak

hydrophobic interactions may form between His194, Leu227, Tyr308, Trp531 and A532 with the

compound (Figure 8B).

Table 4.Potential furin inhibitors from the common antiviral drugs database

No. Drug Name Structure Pharmacological functions

DNA topoisomerase II 1 Suramin inhibitor

Human immunodeficiency 2 Indinavir virus Protease (HIV PR)

Hepatitis C virus Serine

3 Boceprevir protease NS3/4A (HCV

NS3/4A) Modulator

HIV-1 nucleotide reverse 4 Tenofoviralafenamide transcriptase inhibitor

HIV, HBV nucleotide 5 TenofovirDisoproxil reverse transcriptase

inhibitor

Acycloguanosine Thymidine kinase of triphosphate herpesvirus

Hepatitis C virus Serine

Telaprevir protease NS3/4A (HCV

NS3/4A) Modulator

Human immunodeficiency Dolutegravir virus Integrase (HIV IN)

1.C-C chemokine receptor

type 5 (CCR5) Maraviroc 2.CCR5 messenger

RNA(CCR5 mRNA)

Inhibitor of cytochrome Cobicistat P450 3A (CYP3A) enzymes

Nucleoside analogue reverse

transcriptase inhibitor used Stavudine triphosphate in the treatment of HIV

infection

Figure 8.Low-energy binding conformations of Suraminto furin generated by molecular docking. (A) Suraminwas fitted

well in the active pocket of human furin, and furin was shown as electrostatic surface model. Suramin (yellow) was overlapped

with substrate analogue inhibitor MI-52 (purple).(B) Detailed view of Suramin-binding in the activepocket of furin.

4. Discussion Our previous study(accepted by ActaPharmaceuticaSinica B) analyzed the amino acid

composition of the RBD domain of the ACE2 receptor of SARS-CoV-2 and Bat-CoVRaTG13. We

found that several key amino acids determining binding were mutated in SARS-CoV-2, which are

more similar tothat of SARS-CoV.The calculation results show that in the same conformation as

the SARS-CoV protein, the binding energy of SARS-CoV-2 and ACE2 receptors was a litter

higher, but this result cannot fully explain the epidemiologically high contagion, so we speculate

(1)the RBD domain of SARS-CoV-2 may have other conformations; (2) there may be other

receptors; and (3) there are other mechanisms that enhance infectivity. During this manuscript was

prepared, the Cryo-EM structure of SARS-CoV-2 Spike was solved[20]. Comparing thestructure of

SARS-CoV-2 with the Spike structure of SARS-CoV, combined with biophysical detection, they

found that SARS-CoV-2 binds more strongly to cellular ACE2 receptors. Furthermore, the just

disclosed crystal structure of SARS-CoV-2 RBD-ACE2 complex showed a distinct

conformational change in the key loop of complex binding interface. And the binding free energy

calculation indicated a slightly stronger binding for SARS-CoV-2 RBD compared to that of SARS

RBD. These results confirm our guess that the conformational change of the RBD domain of

SARS-CoV-2 leads to stronger binding. However, stronger receptor binding still can' t fully

explain the more infectious problem.

So we put forward these hypotheses: (1) SARS-CoV-2 can also bind to other receptors; (2)

the lung may not be the earliest infection site; (3) SARS-CoV-2 is easier to cut and more easily

fuse with cell membranes. Published in the Pubmed database, researchers performed RNA-seq

analysis on tissue samples from 95 individuals' 27 different tissues. The results showed that ACE2

protein was highly expressed in the small intestine and duodenum, but the expression level in lung

tissue is low (Figure S5). However, we analyzed the expression of furin and found that it is

distributed in various organs with little difference in expression level. Combined with the possible

infection mechanism of SARS-CoV-2, the widespread distribution of furin increases the

SARS-CoV-2 infection of other organs. The possibility of other organ attack is consistent with the

multiple symptoms observed in clinic of COVID-19.

Based on these three conjectures, we compared the Spike sequences from SARS-CoV-2,

SARS-CoV, MERS-CoV and Bat-CoVRaTG13, and found that anextra “PRRA” insert near the

S1/S2 cleavage site. The “PRRA” insert and subsequent arginine (R) constitute a RRAR sequence

that can be recognized and cleaved by furin-like proteases, which may be the reason why

SARS-CoV-2 infection is stronger than SARS-CoV. What’s more, we performed a homologous

alignment and phylogenetic analysis of the SARS-CoV-2 sequence, and found that “PRRA” insert

did not appear at any other close relatives of SARS-CoV-2, indicating that this insertwas

completely novel in this genus virus. The existence of such a motif may allow Spikes to be cut

into S1 and S2 by furin-like proteases before maturity, but not separated, which provides S1 with

the flexibility to change the conformation to better fit the host receptor. According to Simmons G

et al. studies, overexpression of furin can increase the activity of SARS-CoVSpike, but it will not

cause Spike to be cleaved [23].This is consistent with our prediction.

Furthermore, Glowacka Ietal.and Simmons Get al.studies have demonstrated that

SARS-CoVSpikes can be activated by cleavage in two ways, including proteolytic activation by

cathepsins B and L in host cells [24]. In addition, SARS-CoVSpike can be activated by TMPRSS2

cleavage on the host cell surface[6].What’ s more, MERA-CoV, S1/S1 and S2’ cleavage sites cannot

be cut by fruin[25].So we speculated that the activation of SARS-CoV-2 Spike can be through

different protease cleavage pathways and these pathways can occur simultaneously in host cells.

SARS-CoV-2 Spike can utilize host protease diversity to activate, which may explain the strong

infectious capacity of SARS-CoV-2. As we can see in Figure 2, the Spike protein of SARS-CoV-2

can be cleaved at multiple stages, which greatly increases the efficiency of fusion. It is likely that

the virus will fuse with the cell during endocytosis and release the genome. In addition, the

binding ability of the cleaved Spike to the ACE2 receptor is also greatly enhanced [26].

According to our study, furin-like proteases may be potential drug targets for

anti-SARS-CoV-2 treatment. At present, some peptide inhibitors have been developed and have

good effects [27, 28]. To search potential inhibitors of furin-like proteases, we screened potential

compounds from a ZINC drug database (2924 compounds), a small in-house database of natural

products (1066 compounds), and existing antiviral drugs library (78 compounds) withfurinby

virtual ligand screening. From the ZINC Drug Database, we found a series of anti-tumor,

antibacterial, antivirus,hepatoprotective drugs, such as Aminopterin,

Fludarabinephosphate,Sulfoxone,Irinotecan, Hydroxystilbamidine, Lomefloxacin, Cefoperazone,

Valganciclovir,Imatinib, etc. might be used as furin inhibitors. For the natural products, some

flavonoids, diterpenoids, and steroids with antivirus and anti-inflammation effects, such as ECCG,

Biorobin, Phyllaemblicin G7, Andrographolide and its derivatives, and xanthonesfrom the

Swertiagenus, etc.exhibited high binding affinity to furin protein. From the database of 78

antiviral drugs, a series of HIV-1, hepatitis C, and hand-foot-and-mouth disease therapeutic drugs,

such as Indinavir, Tenofoviralafenamide, Tenofovir, Disoproxil, Dolutdegravir, Boceprevir,

Telaprevir and Suraminalso showed high binding affinity to furin. These potentialfurin inhibitors

and medicinal plants containing these compounds as major constituents might be useful for the

treatment COVID-19. The further experiments to verify their efficiency in viro and in vivo will be

carried out in our future studies. What' s more, combined administration of targeting different

SARS-CoV-2 proteases with furin inhibitors may be an effective therapeutic strategy.

ACKNOWLEDGEMENTS

We acknowledge support from National Mega-project for Innovative Drugs (grant number

2019ZX09721001-004-007), National Natural Science Foundation of China (NSFC) (grant

number No.U1803122, 81773637, 81773594, U1703111).

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Furin, a potential therapeutic target for COVID-19

Supplementary information

Figure S1. Multiple sequence alignment of 1000 Spike proteins. These 156 proteins were ranked according to their homology

with SARS-2.The sequence corresponding to PRRA in SARS-CoV-2 in each sequence is marked in the red box.

Figure S2. Result of furin cleavage site pridiction of Spike protein in SARS-CoV-2, which predicted by online method ProP 1.0

Server.

Figure S3. Protein-protein dockingcalculation model of SARS-CoV-2 spike RBD (light blue) with human ACE2 (yellow),

original RBD conformation was shown in orange. The calculated free energy is -50.13 Kcal/mol.

Figure S4. Comparison of SARS-CoV-2 spike RBD (orange) and SARS spike RBD (yellow). The complex with ACE2 (left part,

yellow) was shown. The homology model of SARS-CoV-2 spike RBDbuilt from SARS spike RBD was shown as blue.

Figure S5. Expression levels of Furin, ACE2 and TMPRSS2 in various tissues. The data is from pubmed [1-3].

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