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Original Article

In Silico Screening of Potential Spike Glycoprotein Inhibitors of SARS-CoV-2 with Drug Repurposing Strategy*

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ABSTRACT **Objective:** To select potential molecules that can target viral spike proteins, which may potentially interrupt the interaction between the human angiotension-converting enzyme 2 (ACE2) receptor and viral spike protein by virtual screening. **Methods:** The three-dimensional (3D)-coordinate file of the receptor-binding domain (RBD)-ACE2 complex for searching a suitable docking pocket was firstly downloaded and prepared. Secondly, approximately 15,000 molecular candidates were prepared, including US Food and Drug Administration (FDA)-approved drugs from DrugBank and natural compounds from Traditional Chinese Medicine Systems Pharmacology (TCMSP), for the docking process. Then, virtual screening was performed and the binding energy in Autodock Vina was calculated. Finally, the top 20 molecules with high binding energy and their Chinese medicine (CM) herb sources were listed in this paper. **Results:** It was found that digitoxin, a cardiac glycoside in DrugBank and bisindigotin in TCMSP had the highest docking scores. Interestingly, two of the CM herbs containing the natural compounds that had relatively high binding scores, *Forsythiae fructus* and *Isatidis radix*, are components of Lianhua Qingwen (莲花清瘟), a CM formula reportedly exerting activity against severe acute respiratory syndrome (SARS)-Cov-2. Moreover, raltegravir, an HIV integrase inhibitor, was found to have a relatively high binding score. **Conclusions:** A class of compounds, which are from FDA-approved drugs and CM natural compounds, that had high binding energy with RBD of the viral spike protein. Our work provides potential candidates for other researchers to identify inhibitors to prevent SARS-CoV-2 infection, and highlights the importance of CM and integrative application of CM and Western medicine on treating COVID-19.

KEYWORDS COVID-19, SARS-CoV-2, drug repurposing, virtual screening, Chinese medicine

Since the middle of December 2019, a novel coronavirus disease (COVID-19) outbroke, and rapidly spread throughout the world. Early studies utilized real-time reverse transcription-polymerase chain reaction (RT-PCR) to confirm that the samples collected from one patient with pneumonia were positive for pan-betacoronavirus.[1] The viruses isolated from human cell lines were observed to contain typical crown-like shapes under a transmission electron microscope (TEM) with negative staining.[1] Based on metagenomic sequencing, the whole-genome sequence of the virus was determined.[1,2] Bioinformatics analysis indicated that the novel virus causing severe pneumonia is a new type of betacoronavirus. The virus shares various typical genomic compositions with other betacoronavirus family members and has the highest sequence homology (96% sequence similarity) to severe acute respiratory syndrom (SARS)-like RaTG13 in bats,[1] and the virus is named as SARS-CoV-2.

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To date, COVID-19 cases have been reported in more than 200 countries. As of July 20, 2020, a cumulative total of 14,340,653 COVID-19 cases were reported worldwide with 603,111 death. Although viral nucleic acid detection kits have been rapidly developed, effective drugs and vaccines are still being studied and are urgently needed. Facing such urgent situations, although necessary, developing new drugs or vaccines will require a very long time to complete, and drug repositioning is more rapid and possible to discover anti-SARS-CoV-2 drugs from US Food and Drug Administration (FDA)-approved drugs.[3]

Chinese medicine (CM) has been applied in combating virus-related diseases such as SARS and H1N1[4] and a large number of studies indicated the therapeutic effects of integrative application of Western medicine (WM) and CM were significantly better than the simple application of WM.[5-10] Essentially, although the therapeutic components are still unclear, Lianhua Qingwen (连花清瘟),[11] Toujie Quwen Granule (透解祛瘟颗粒), and Qingfei Paidu Decoction (清肺排毒汤) are playing an important role in the treatment of COVID-19.[12,13] Thus, rapid screening from these sources is a promising approach for discovering anti-COVID-19 drugs.[4,14]

Receptor recognition by coronavirus is the first and essential step for entering human cells. The homotrimeric spike glycoprotein (S protein), located on the envelope of SARS-CoV-2, is responsible for receptor recognition. The S1 subunit of the S protein, containing the receptor-binding domain (RBD), directly interacts with the receptor on the human cell membrane, while the S2 subunit facilitates virus-cell fusion and entry.[15] Angiotensin-converting enzyme 2 (ACE2) was shown to be the receptor mediating SARS-CoV-2 invasion in human cells.[1] Thus, interrupting the interaction between the S protein and ACE2 is a strategy to inhibit virus entry.[16]

Recently, three-dimensional (3D) structures of the homotrimeric spike glycoprotein[17] and the ACE2-B0AT1 complex[18] were solved by cryo-EM respectively. In addition, the complex structures of the receptor-binding domain (RBD) of the S protein bound to the human ACE2 were independently reported by several research teams.[19-21] These accurate coordinates of protein structures make it possible for avoiding inaccurate protein structure modeling and accelerating drug screening and vaccine design.

Here, we performed the structure-based virtual screening to search for molecules from the DrugBank and Traditional Chinese Medicine Systems Pharmacology (TCMSP) databases to identify potential inhibitors targeting the RBD of viral spike proteins. The selected binding pocket neighbors the interface between the viral spike protein and human receptor ACE2, which may further inhibit their interaction to prevent the virus from invading human cells. Following this computational work, more vigorous *in vitro* and *in vivo* experiments need to be performed. We hope this work will contribute to the discovery of anti-COVID-19 drugs.

METHODS

DrugBank is a drug data resource that contains comprehensive drug information, including approved and experimental small-molecule, biologics, and nutraceutical data.[22] The latest version of DrugBank was released on January 13, 2020. It currently has 2,628 approved small drugs. Before performing virtual screening, approved drugs with molecular weights larger than 500 kDa, including polypeptides, were deleted; thus, only 2,191 FDA-approved drugs were screened. TCMSP was developed by Northwest University in China, which is a platform providing information connecting bioactive molecules in CM, targets, and diseases in a network. In addition, this platform also provides related pharmacokinetic properties.[23] Based on this platform, 13,026 small molecules from CM herbs were screened. All molecular structure files were optimized by the force field MMFF94.

AutoDock Vina 1.1.2[24] was used in this work to perform virtual screening with the Lamarckian genetic algorithm as a scoring function. We only set exhaustiveness as 20 to perform docking. For the other parameters of Autodock vina, their default values were used. The docking of Autodock vina was performed at the given coordinates. PyMOL 2.3.3[25] and Discovery Studio 2016[26] were used to exhibit the ligand-receptor interactions. The 2.5Å-resolution crystal structure of the RBD of the spike protein complexed with the ACE2 receptor was published on the National Microbiology Data Center (No. NMDCS0000001; PDB ID: 6LZG).[27] According to this crystal structure, Discovery Studio 2016 was employed to detect the binding pocket on the RBD. Both Discovery Studio 2016 and Molecular Operating Environment (MOE) 2019 were used to choose the binding site on S protein.

AMBER16 was employed to perform molecular dynamics (MD) simulation. The MD simulation is described as follow. The complex was neutralized by adding sodium/chlorine counter ions and solved in a cuboid box of TIP3P water molecules with solvent layers 10 Å between the box

edges and solute surface. The AMBER FF14SB force fields were applied and the SHAKE algorithm was used to restrict all covalent bonds involving hydrogen atoms with a time step of 2 fs. The particle mesh Ewald method[28] was employed to treat long-range electrostatic interactions. For each solvated system, two steps of minimization were performed before the heating step. The first 4,000 cycles of minimization were performed with all heavy atoms restrained with the force constant 50 kcal/(mol·Å^2), whereas solvent molecules and sodium/chlorine counter ions were free to move. Then, non-restrained minimization was carried out involving 2,000 cycles of steepest descent minimization followed by 2,000 cycles of conjugated gradient minimization. Afterwards, the whole system was first heated from 0 to 300 K in 50 ps using Langevin dynamics at a constant volume and, then, equilibrated for 400 ps at a constant pressure of 1 atm. A weak constraint of 10 kcal/(mol·Å^2) was used to restrain all the heavy atoms during the heating steps. Periodic boundary dynamics simulations were carried out for the whole system with an NPT (constant composition, pressure and temperature) ensemble at a constant pressure of 1 atm and 300 K in the production step. In production phase, 100 ns simulation was carried out. The binding free energy of complex was calculated using the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) method.[29]

RESULTS

Binding Pocket Identified on RBD of Spike Protein

The second-ranked pocket (called Site 2) was selected as the drug target for further ligand docking because it is near the protein-protein interface and may thus induce a conformational change to intervene in the interaction (Figure 1). Site 2 actually has the highest score among the 3 sites. The coordination of the pocket is as follows: center x=–56.387, center y=52.408, center z=21.937; size x=25, size y=25, size z=25.

Docking Results of Molecules from DrugBank Dataset

For the DrugBank dataset, Table 1 lists the top 10 compounds with the highest binding energy with Site 2. The specific binding score results for each compound are available upon request.

The compound digitoxin was identified as having the lowest binding energy, –8.7 kcal/mol, at the binding site. Figures 2A–2D shows the interaction of digitoxin with RBD of the S protein. Here, digitoxin formed five

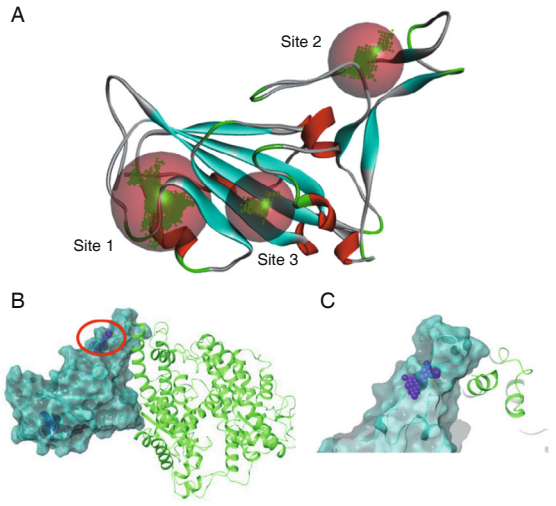

Figure 1. Binding Pocket Identified on Receptor-Binding Domain of Spike Protein

Notes: (A) Top 3 pockets predicted by Discovery Studio 2016. The pockets are labeled according to their rank. (B) The position circled in red is the pocket selected. The protein colored green is ACE2. (C) The selected site is zoomed in. The position consisting of purple balls is the pocket selected

conventional hydrogen bonds with LYS458, SER459, ASP467, GLU471, and 3 carbon-hydrogen bonds (non-classical hydrogen bonds) with LYS458 and GLU471. Also, digitoxin formed alkyl hydrophobic interaction with LYS458 and PRO491. Digitoxin is a cardiac glycoside. It has a known target, the sodium/potassium-transporting ATPase subunit. Digitoxin can be used to treat patients with heart failure. Scientists have found that digitoxin is also a potential anticancer drug.[30,31] However, digitoxin can cause toxicity to the human body, including causing nausea, anorexia, confusion and so on.

Docking Results of TCMSP Dataset

The top 10 binding results of the TCMSP dataset are shown in Table 2. The complete binding scores information of TCMSP are available upon request.

The compound with the highest binding energy in the TCMSP dataset with the S protein is bisindigotin, which had –8.3 kcal/mol. Figures 2E–2H shows the interaction between bisindigotin and RBD of the S protein. Here, bisindigotin formed 3 hydrogen bonds with ARG457, SER469 and GLU471 and one carbon-hydrogen bond with LYS458. The electrostatic interaction promoted the binding of bisindigotin with Site 2 by forming Pi-Anion interaction with ASP467 and GLU471. And bisindigotin formed hydrophobic Pi-Alkyl interaction with ARG457 as well. Intriguingly, bisindigotin can be isolated from *Isatis indigotica* and *polygoni tinctorii toliu*. Both herbs have heat-clearing and detoxifying effects in CM theories.

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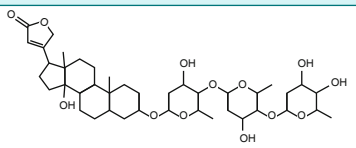
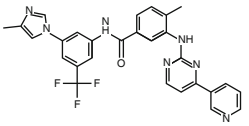
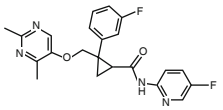
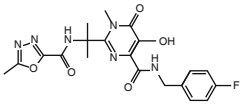
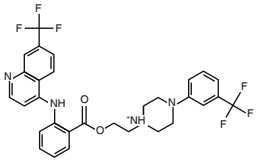
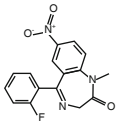
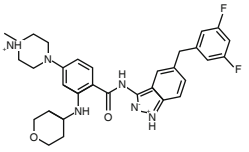
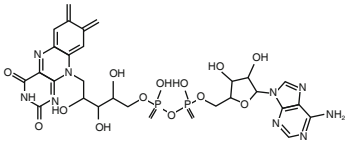
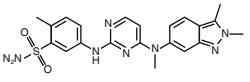
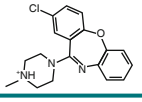
Table 1. Top 10 RBD-Binding Molecules from DrugBank

DrugBank ID	Name	Score (kcal/mol)	Structure	Original disease
DB01396	Digitoxin	–8.0		Congestive cardiac insufficiency, arrhythmias, and heart failure
DB04868	Nilotinib	–7.9		Leukemias
DB11951	Lemborexant	–7.7		Difficulties with sleep onset and/or sleep maintenance
DB06817	Raltegravir	–7.5		HIV infection
DB01419	Antrafenine	–7.4		Anti-inflammatory and analgesic agent
DB01544	Flunitrazepam	–7.4		Severe insomnias
DB11986	Entrectinib	–7.4		Non-small-cell lung cancer
DB03147	Flavin adenine dinucleotide	–7.3		Vitamin B2 deficiency
DB06589	Pazopanib	–7.3		Advanced renal cell cancer and advanced soft tissue sarcoma
DB00408	Loxapine	–7.3		Psychotic disorders, including schizophrenia

Lsatis indigotica is a folk medicine used to treat viral disease and inflammatory disease.[32] In addition, Wei, et al[33] reported that bisindigotin can act as an antagonist to relieve the hepatoxicity caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin, which is a carcinogen.

Molecular Dynamics Simulation

The binding energy between digitoxin and S protein was –65.191 ± 18.935 kJ/mol (–15.571 ± 4.523 kcal/mol). The result of RMSD also reflect that the docking between digitoxin and S protein is stable (Figure 3). In addition, 100-ns MD simulation was also performed to verify the reliability of binding between bisindigotin and viral S protein. However, the result was not convergent.

DISCUSSION

The world is now confronted with a public health crisis caused by SARS-CoV-2. Normally, *de novo* drug development is a time-consuming project. Therefore, drug repurposing may be helpful to find the drug

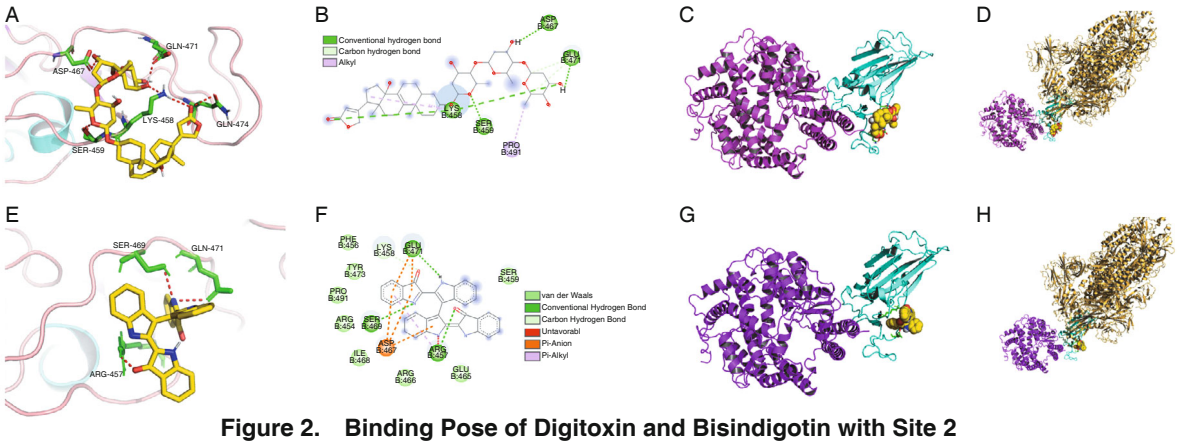


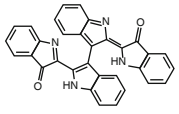
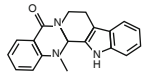
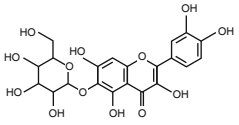
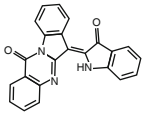
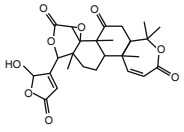
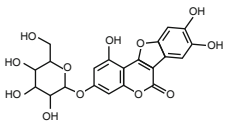
Figure 2. Binding Pose of Digitoxin and Bisindigotin with Site 2

Notes: (A) and (E) show the 3D interactions of digitoxin and bisindigotin, respectively, with Site 2 (green sticks, pink loops, and cyan helix). The red dotted lines represent polar contacts. Digitoxin and bisindiotin are colored yellow. (B) and (F) show the 2D interactions of digitoxin and bisindigotin, respectively, with RBD of the S protein. (C) and (G) present digitoxin and bisindigotin, respectively, on the interface between the RBD of the S protein and ACE2. The cyan part is the S1 subunit of the S protein. The yellow, red, white balls represent digitoxin or bisindigotin; the protein colored magenta is ACE2. (D) and (H) show digitoxin and bisindigotin, respectively, on the interface between the S protein and ACE2. The protein colored magenta is ACE2; the cyan part represents the S1 subunit of the S protein. The yellow, red, white balls represent digitoxin or bisindigotin. The trimer S protein (golden, PDB ID: 6VSB) is superposed on the RBD-ACE2 complex (PDB ID: 6LZG)

preventing coronavirus as soon as possible.

Here, we firstly chose the spike protein rather than the ACE2 receptor for a binding pocket search because ACE2 is expressed in various types of human cells, as a result, targeting ACE2 might cause more side effects. We observed that the cavity neighboring the interface between the viral spike protein and the human ACE2 receptor is the second-ranked pocket. Targeting this position may contribute to interrupting the interaction between the S protein and the ACE2 receptor. Due to its high ranking and position advantages, we used this cavity for the next

Table 2. Top 10 RBD-Binding Molecules from TCMSP

TCMSP ID	Name	Score (kcal/mol)	Structure	CM herb
MOL011100	Bisindigotin	–8.3		*Polygoni Tinctorii Foliu* (蓼大青叶), *Indigo Naturalis* (青黛)*
MOL003958	Evodiamine	–8.1		*Evodiae Fructus* (吴茱萸)
MOL002729	Quercetin-6-glucoside	–8.0		*Carthami Flos* (红花)
MOL001810	6-(3-oxoindolin-2-ylidene) indolo(2,1-b)quinazolin-12-one	–7.9		*Isatidis Radix* (板蓝根)*, *Isatidis Folium* (大青叶), *Indigo Naturalis**
OL002659	Kihadanin A	–7.8		*Phellodendri Chinrnsis Cortex* (黄柏)
MOL003377	Demethylwedelolactone-7-glucoside	–7.8		*Ecliptae Herba* (墨旱莲)

(To Be Continued)

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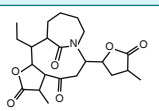
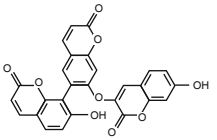
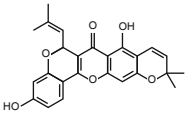
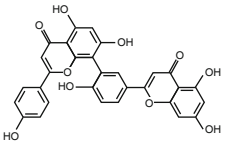
(Continued)

TCMSP ID	Name	Score (kcal/mol)	Structure	CM herb
MOL009370	Tuberostemonone	–7.8		*Stemonae Radix* (百部)*
MOL011124	Wikstrosin	–7.7		*Wikstroemiae Indicae Rasix* (了哥王根)*
MOL000743	Cyclomulberrochromene	–7.6		*Ramulus Mori* (桑枝)
MOL002037	Amentoflavone	–7.6		*Ginkgo Semen* (白果)*, *Platycladi Cacumen* (侧柏叶)*, *Herbahypericiperforati* (贯叶金丝桃), *Herba Selaginellae Moellendorfii* (卷柏), *Forsythiae Fructus* (连翘)*, *Ranunculi Ternati Radix* (猫爪草)*, *Canarii Fructus* (青果)*, *Selaginella Doederleinii Hieron* (石上柏)*, *Ginkgo Folium* (银杏叶)*

Note: *In CM, these herbs have effects on clearing heat-toxin and are usually utilized to relieve symptoms of pneumonia

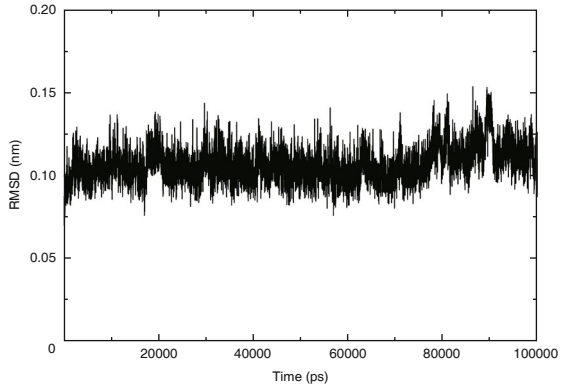


Figure 3. RMSD Results between Digitoxin and S Protein

docking progress. On the other hand, future work might focus on identifying a suitable cavity within the interface formed by the viral spike protein and human receptor ACE2.

In this work, two databases, DrugBank, containing FDA-approved drugs, and TCMSP, containing CM bioactive molecules, were used to explore potential candidates to prevent SARS-CoV-2 from invading the human body. The docking results show that the molecules having the lowest binding energy from DrugBank and TCMSP are digitoxin and bisindigotin, respectively. Bisindigotin is a bioactive CM from *Isatis indigotica*. This plant has been used as an herb in China to treat viral disease.

Additionally, in the top-10 list of DrugBank docking results, it is worth knowing that an HIV antiretroviral drug, raltegravir, was identified, which is a new class of HIV drugs that can inhibit HIV integrase. Considering other HIV inhibitors such as lopinavir and ritonavir have been proved to have anti-coronavirus effects, raltegravir might be worthy of paying more attention.

On the other hand, in the top-10 list of TCMSP, we observed that many herbs contain molecules that have effects on clearing heat-toxin, which include *Indigo naturalis*, *Isatidis radix*, *Isatidis folium*, *Stemonae radix*, *Wikstroemiae indicae rasix*, *Ginkgo semen*, *Platycladi cacumen*, *Forsythiae fructus*, *Ranunculi ternati radix*, *Canarii fructus, Selaginella doederleinii hieron*, and *Ginkgo folium*. According to CM theories, these herbs can be used to relieve symptoms of pneumonia. Importantly, *Forsythiae fructus* and *Isatidis radix* were main components of Lianhuaqingwen, a widely used CM in treating COVID-19 patients in China, and were issued in the 7th Guideline for the Diagnosis and Treatment of Novel Coronavirus (2019-nCoV) Pneumonia published by National Health Commission of the People's Republic of China.[13]

Taken together, this study provides other researchers with potential inhibitors that inhibit the interaction between spike protein and human ACE2. Meanwhile, this study supports the clinical application of *Forsythiae fructus* and *Isatidis radix* in treating COVID-19. These results indicate that the combination of CM and WM could be a promising

approach to treat COVID-19 disease. In the future research, *in vitro* tests will be carried out to verify the binding between the S protein and the molecules identified by the computational methods as well as their inhibitory abilities against the interaction between RBD and ACE2 receptors.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Li HY, Wang GY and Zhang J conceived and designed the study. Wei TZ and Wang H performed the research, and contributed to this work equally as co-first authors. All the authors analyzed the data. Li HY, Wang GY, Zhang J, WEI TZ, and Wang H wrote the manuscript draft. All the authors read and approved the final version of manuscript.

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