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Feasibility and Effective Binding Inhibitors against COVID-19 BA.2.86

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- Molecular interaction energy
- Binding inhibitor
- Cocktail dose

Abstract

The binding strength of COVID-19 variants, Omicron BQ.1, XBB.1.5, XBB 1.16, FE.1, EG.5, and BA.2.86, with the ACE-2, was calculated and evaluated with previous variants. The binding inhibition of various compounds (including PF07321332, Molnupiravir, and Xocova) was investigated. The binding strength of BA.2.86 with ACE-2 was similar to that of Delta, and the binding strength of the others was weak. Several medicines inhibited binding Delta S-RBD with ACE-2, but not that of BA.2.86. The basic amino acid R454 is buried in Delta S-RBD, but that of BA.2.86 is free for binding with ACE-2. The selectivity encourages the further designing of medicines. These medicines did not inhibit the binding of BA.2.86, but their malic acid complexes inhibited the binding. If these compounds inhibit the multiplication, their cocktails with TCA-cycle acids should effectively inhibit the binding.

INTRODUCTION

Three years after COVID-19 was recognized as a pandemic disease, there is a persistent and considerable burden of symptoms and multi-system organ involvement in an important subgroup of people. We are going to need many years of careful follow-up to understand the ways and extent Covid has hurt us fully. We must consider finding effective ways to treat people who suffer from COVID-19 as an urgent and foremost priority [1]. Numerous sublineages emerged from the continued evolution of the SARS-CoV-2 Omicron variants with different patterns of evasion from neutralizing antibodies. Various facts were observed, such as human leukocyte antigen (HLA) class 1 and class 2 resented T-cell epitopes in the spiked-glycoprotein were highly conserved across the entire evolution of SARS-CoV-2 suggesting that CD8+ and CD4+ T-cell recognition of Omicron, BQ.1.1, BA.2.75.2 and XBB might be largely intact. It is considered that Omicron sublineages effectively evaded B-cell immunity by altering neutralizing antibody epitopes [2], and *N*-acetyl cysteine covalent conjugation perturbed the stereo- specific orientations of the interacting key residues of spike protein, resulting in a threefold weakening in the binding affinity of spike protein with the ACE-2 receptor [3]. Persistent SARS-CoV-2 RNA was detected in multiple anatomic sites throughout the brain [4]. SARS-CoV-2 non-structural protein 1 was sufficient to confer resistance to Natural killer cell killing [5]. Coldspot-guided antibody discovery reveals donor-derived neutralizing antibodies that were cross-reactive with Orthocoronavirine, including SARS-CoV-2 variants [6]. T cell epitope mapping across the SARS-CoV-2 proteome would allow us to understand better the risks presented by merging SARS-CoV-2 variants and the mechanisms that drove viral evolution [7]. Adding adjuvants or changes in doses and more mechanistic interventions might be implemented, such as using IL-7 [8].

Various side effects of vaccination have been reported: capillary leak syndrome [9] and vaccine-induced myocarditis [10]. Furthermore, free spike antigen was detected in the blood of adolescents and young adults who developed post-mRNA vaccine myocarditis [11]. Fragment crystallizable-mediated effector functions are critical for antiviral immunity; therefore, the choice and timing of vaccination regimens using mRNA vaccines against SARS-CoV-12 because the induction of IgG4 antibodies was not observed after homologous or heterologous SARS-CoV-2 vaccination with adenoviral vectors [12]. Live vaccines induce positive non-specific effects, whereas non-live vaccines induce several negative non-specific effects. The non-specific effects of mRNA vaccines on overall mortality should be studied in depth [13]. Therefore, there was a proposal that we should stop trying to prevent all symptomatic infections in healthy young people by boosting them with vaccines containing mRNA from strains that might disappear a few months later [14].

Further details of variants have been explored. EG.5.1 and BA.2.86 sub-variants exhibited an attenuated replication in hamsters' lungs as compared to the BA.5 variant [15]. The XBB sub-variants showed enhancing transmissibility and ability to escape the adaptive immune response. The infectivity of the BA.2.86 and EG.1 sub- variants was high compared to the BA.5, and the infectious virus titered in the lungs of EG.5.1- and BA.2.86- infected animals were significantly lower compared to the BA.5-infected ones. The lung pathology scores of animals infected with EG.5.1 and BA.2.86 were also markedly lower than that of the BA.5 sub-variant [16]. The relative reproduction number of BA.2.86 is significantly higher than that of EG.5.1. The fusogenicity of the

BA.2.86 spike is similar to that of the parent BA.2 spike, the intrinsic pathogenicity of BA.2.86 in hamsters was significantly lower than that of BA.2. The growth kinetics of BA.2.86 are also significantly lower than that of BA.2 both in vitro and in vivo. The attenuation pathogenicity of BA.2.86 is likely due to its decreased replication capacity BA.2.86 was more transmissible than EG.5.1, even if the sensitivity of BA.2.86 to antiviral drugs was comparable to that of EG.5.1. The replication efficiency of BA.2.86 *in vitro* and *in vivo* was lower than that of EG.5.1 [17].

The BA.2.86 variant appeared, and the effectiveness of a new monovalent XBB.1.5 vaccine showed good levels of neutralizing antibodies [18]. The mutation of COVID-19 was too fast and caused a pandemic environment in our social system; following this, the analysis of transmissibility and estimation of the multiplication was delayed. Preventing infection was an urgent subject. Quickly developed vaccines cannot inhibit the infection. The vaccines were considered to protect patients; however, they have produced different problems. Therefore, we should find an effective way to use available medicines to treat patients. Here, the different capabilities of medicines against Delta and BA.2.86 were analyzed.

The molecular recognition of proteins is based on the selective molecular recognition of proteins. The strongest interaction is ion-ion interaction following hydrogen bonding, then hydrophobic interaction. The quantitative explanation method of molecular interactions was achieved based on a reproducible experiment, liquid chromatography with the stable bonded phase silica gels developed. The mechanism was first explained by enantiomer recognitions that required molecular interaction with steric hindrance. The fundamental method was first applied for quantitative analysis of enzyme reactivities and was furthermore applied for protein-protein interactions. The developed method has been used for quantitative analysis of COVID-19 transmissibility and designing the binding inhibitors [19].

EXPERIMENTAL

The experimental method was the same as that performed previously [20,21]. Replaced amino acid of wild-type S-RBD and optimized the structures. The replaced amino acids are listed in Table 1. Then, the S-RBD mutants faced the extracted ACE-2

Table 1 Mutated S-RBD amino acids

Variants		Mutated amino acids
Delta	L452R, T4478K	K444T, N460K
Omicron	BQ.1	N460K, F486P, F490S
Omicron	XBB.1.5	T478R, F486P
Omicron	XBB.1.16	F456L, F486P, F490S
Omicron	FE.1	F456L, N460K, S486P, FS490S
Omicron	EG.5	I332V, D339H, R403K, V445H, G446S, N450F,
Omicron	BA.2.86	L452W, N481K, 483del, E484K, F486P

and optimized the structure to obtain the molecular interaction (MI) energy values between S-RBD and ACE-2. Acidic medicines targeted to inhibit enzyme activity are selective medicines compared to basic medicines targeted to bind phosphate of DNA and RNA; therefore, basic medicines are less selective.

RESULTS AND DISCUSSION

Previously, binding inhibitor candidates were searched among 82 compounds. The 55 carboxy compounds were repulsed from the ACE-2 binding site where acidic amino acids are lined [20]. The feasibility of these acidic compounds-binding inhibition was analyzed using the Delta variant. For example, an inhibitor candidate lactic acid was bound on the S-RPD binding site, as shown in Figure 1, where three lactic acids were used to cover the S-RBD binding site. The complex of Delta S-RPD and three lactic acids was located under the ACE-2 and optimized the conformation. The complex and the ACE-2 bound together. The lactic acid did not inhibit the binding. This is a pharmacological mistake. Lactic acid is ionized in physiological conditions. Therefore, ionized lactic acid should be used for the complex formation and binding inhibition study. Three ionized lactic acids were bound with Delta S-RPD, and the complex was located below the ACE-2 and optimized the conformation. Figure 2 shows that the ionized lactic acid inhibited the binding. This type of mistake was found in reports where an auto docking program was introduce.

Other inhibitors are ionized m-crboxyl-*L*-tyrosine, m-carboxyl-*L*-tyrosine *N*-mannoside, citric acid, citric acid dimethyl, citric acid *O*-mannoside, citric acid di-*O*-mannoside, Ferulic acid, Gallic acid, Ibuprofen, Glycyrrhizinic acid, mefenamic acid, nalidixic acid, naproxen, probenecid, and Cyonic acid. The residual amino acid of Delta S- RBD is K478, which binds one of these carboxy groups. These ionized form compounds inhibited the binding but not their molecular form compounds. The process of these compounds is shown in Figure 3.

The fundamental stereo structure of the protein and a medicine (substrate) for two medicines, PF-07321332 (Nirmatrelvir /Ritonavir/ Paxlovid) and Molnupiravir (RIDD2801), was reported; therefore, we can compare the stereo structure of other medicines whether other medicines can replace from these medicines. Therefore, the properties of many medicines are used now by docking with S-RBD, and the medicine S-RBD complex docking with ACE-2 actual site. Medicines that have been officially

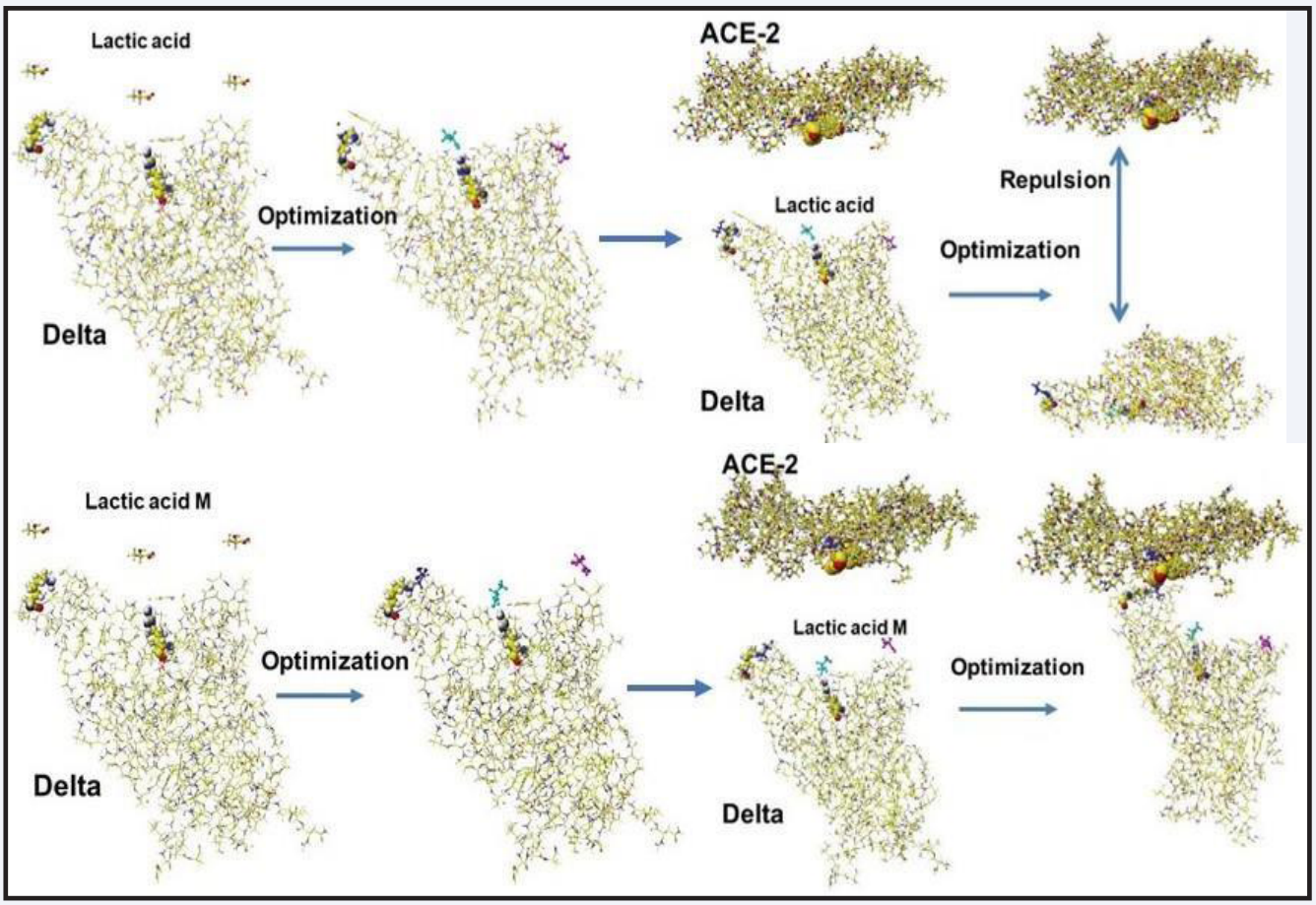


Figure 1 Properties of docking inhibitor candidate molecular form lactic acid.

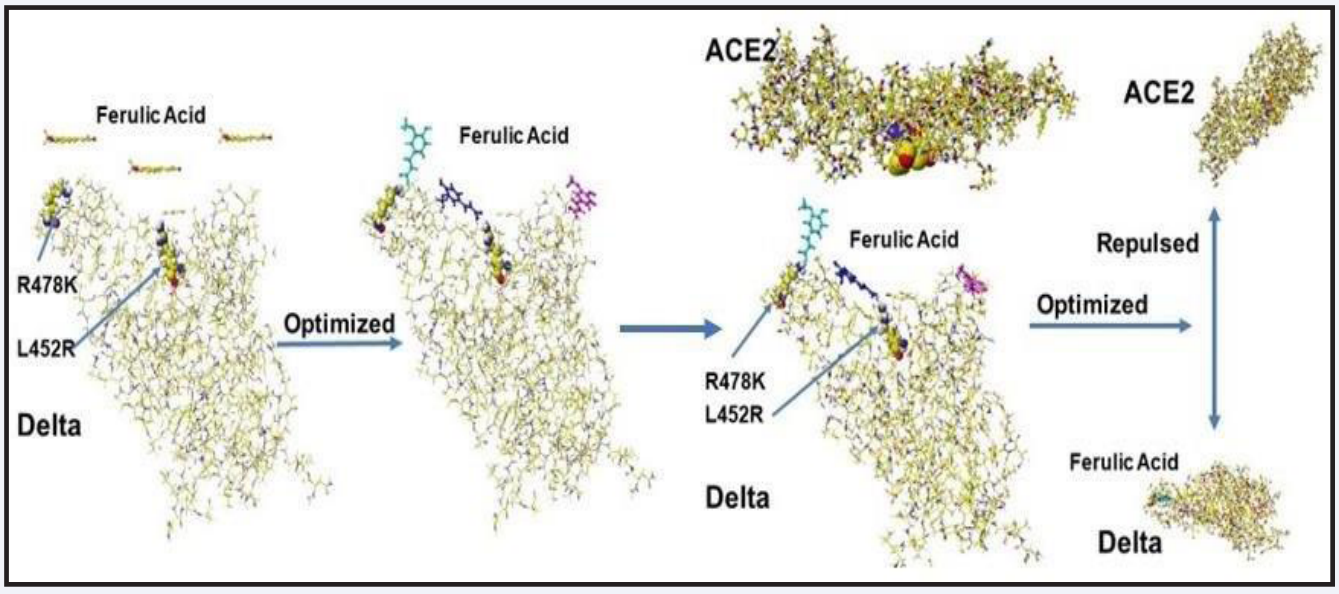


Figure 2 Properties of docking inhibitor candidate ionized form lactic acid.

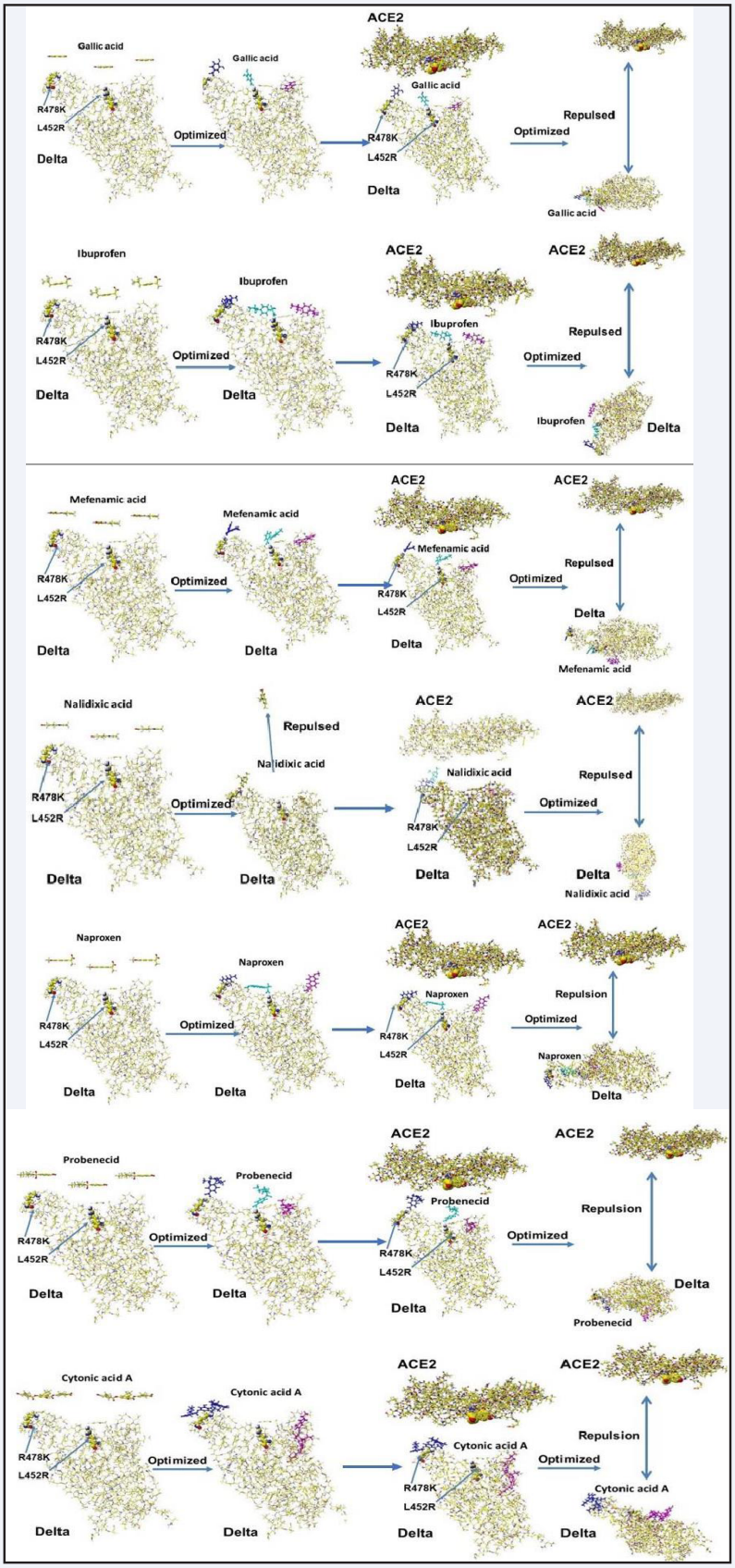


Figure 3 Performance of acidic compounds nding inhibition.

used to treat COVID-19 patients are PF07321332 (Nirmatrelvir), Molunupiravir, and Xocove (Ensitrelvir Fumarate). However, PF07321332, Molnupiravir, and the original Xocoba did not inhibit the binding shown in Figure 4.

The same analysis was performed for the Omicron BA.2.86 variant. The mutated amino acids of S-RBD are D339H, R403K, V445H, G446S, N450D, L452W, N481K, E484K, and R486P, and the mutation was complicated compared to the mutation of the Delta, whose spike mutations of interest were L452R, T478K, D614G, and P681R. The key amino acids for binding of BA.2.86 are R403K, N481K, and E484K. Especially, E484K enhances the binding. The binding strength was XBB1.5 (373.9 kcal mol^{-1}) << BA.2.86 (556.2 kcal mol^{-1}) < Delta (594.2 kcal mol^{-1}) < BA.1 (761.7 kcal mol^{-1}) << BA.2 (904.3 kcal mol^{-1}) based on the MI energy values. However, many acidic compounds inhibited the binding of the Delta and the ACE-2 but failed to inhibit the binding of BA.2.86 and the ACE-2, except Glycyrrhizic and Crebs cycle acids.

The possible reason why acidic compounds inhibited Delta S-RBD and ACE-2 binding did not inhibit BA.2.86 and ACE-2 binding is the conformation of R454. Delta R454 is buried inside, but BA.2.86 R454 is located in a relatively open space; therefore, the R454 guanidyl group can bind with ACE-2. Such steric hindrance contributes to binding. Computational chemistry can analyze chemical interaction quantitatively, but it is difficult to analyze interaction.

In physiological conditions, the carboxy group and the polyphenol phenolic hydroxy group are ionized, and these ionized acidic groups effectively inhibit the binding. The ionization of a phenolic hydroxy group can be estimated from the atomic partial charge values (unit: au) calculated by the MOPAC AM1 program. The failed conformations to inhibit the binding of BA.2.86 S-RBD and ACE-2 are not due to the ion-ion interactions between the inhibitor candidates and ACE-2. The Osertrivir amino group is bound with a carboxy group of ACE-2 D38 and E329. It also bound peptide bridge carbonyl via hydrogen bond. Cytonic acid phenolic hydroxy group forms hydrogen bonds with both D36 carboxy and K353 amino groups. One Gallic acid contributes to the binding where a hydroxy group binds with the Q38 amino group. The other two Gallic acid hydroxy groups did not involve the binding. The Furosemide amino group binds with E35 and D38 carboxy groups via hydrogen bonding, and the ferulic acid phenolic hydroxy group binds to the D38 carboxy group. The last three compounds did not inhibit the binding, but these compounds mainly bound to ACE-2 via hydrogen bonding. The results indicated that these acidic compound carboxy groups bound tightly with the BA.2.86 S-RBD via ion-ion interactions, and the binding to ACE-2 was weak hydrogen bonding. That is, the steric hindrance is an important factor of the protein substitute affinity.

Modification of PF07321332 and Molnuporavir

The analysis of binding inhibitor candidates from binding strength with ACE-2 indicates that carboxy compounds were repulsed from the ACE-2 binding site where acidic amino acids line up; therefore, further modification of medical treatment candidates may produce an effective binding inhibitor. The modified PF-07321332, whose cyano group was replaced with

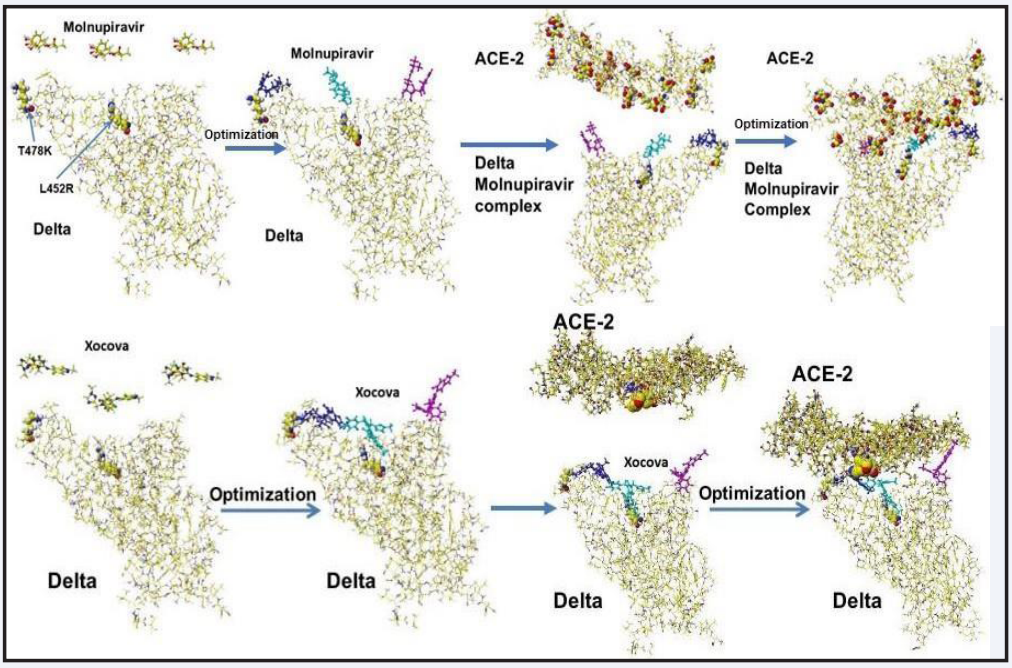


Figure 4 Initial conformations of Delta S-RBD with three PF 07321332 molecules, three molnupiravir molecules, and three Xocova molecules, the complex (optimized) structure of Delta S-RBD with three PF 07321332 molecules, three molnupiravir molecules, and three Xocova molecules complex and ACE-2 and their optimized structure.

a carboxy group based on precursor search in organic reaction, rejected the binding from ACE-2. The chemical structure of PF07321352 and the modified compound is shown in Figure 5.

The same binding analysis was performed for modified PF 07321332. The initial conformation of Delta S-RBD and three modified PF 07321332 molecules, their complex structure, and Delta S-RBD, modified PF 07321332 complex, and ACE-2 and their optimized structures are shown in Figure 6.

The original PF 07321332 and Delta S-RBD complex was bound with ACE-2, but the modified PF 7321332 and Delta S-RBD complex was repulsed from ACE-2. The modification seems to be a promised approach for the effective use of PF 07321332. The same approach was performed for modified Molnupiravir.

The chemical structures of Molnupiravir and the modified compound are shown in Figure 5. Molnupiravir was modified; the hydroxy-amino group was replaced with a carbamate. The three original and modified Molnupiravir molecules are located about 10Å above the Delta S-SBD binding site, then optimized these complexes. Molnupiravir bound ACE-2 structure is shown in Figure 7. These complexes were located about 10Å below the part of ACE-2 and optimized these complexes. The complex with the original Molnupiravir bound with ACE-2 and Molnupiravir did not inhibit the binding, but the modified molnupiravir inhibited the binding, as shown in Figure 7.

Practical use of known medicines as cocktail medicines

Omicron BA.2.86 is a current VOC. The binding strength is about the Delta but below that of Omicron BA.1 and 2. However, the binding strength with ACE-2 is still stronger than previous Omicron BA.4 and 5 variants. The key mutations of Omicron BA.2.86 are N481K and E484K among I332V, D339H, R403K, V445H, G446S, N450F, L452W, and F486P. R403K reduces the binding strength, but E484K (replacing an acidic amino acid with a basic amino acid) strengthens the binding. Therefore, the property of six medicine candidates was investigated. The selected compounds were Chloroquine, Dalcetrapib, Deguetin, Dolutegravir, Ebselen, and Etravine. The *in silico* analytical method is the same as that previously described. Figure 8 shows their structures.

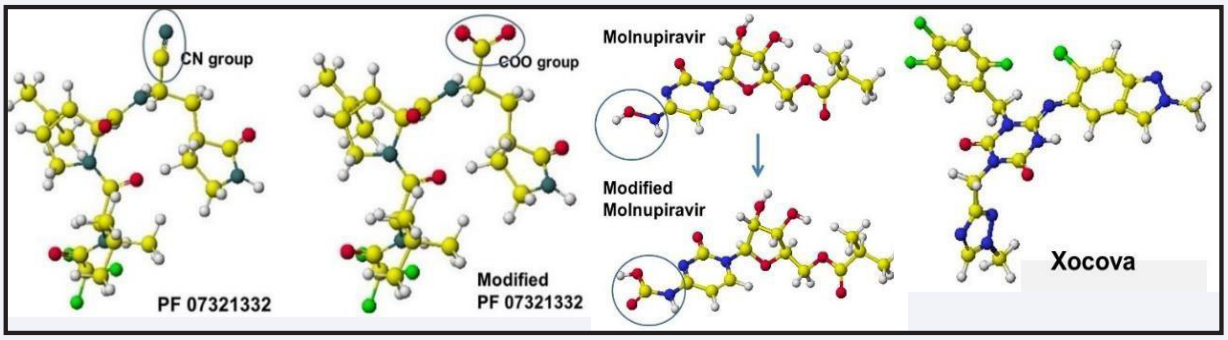


Figure 5 Original and modified stereo structure of PF 07321332, Molnupiravir, and Xocova where atoms are indicated as color or grayscale; nitrogen (blue or dark gray balls), oxygen (red or black balls), carbon (yellow or gray balls), hydrogen (small white balls), and fluorine (light green of light gray balls). Generally, fluorone atoms support the sterically stable structure..

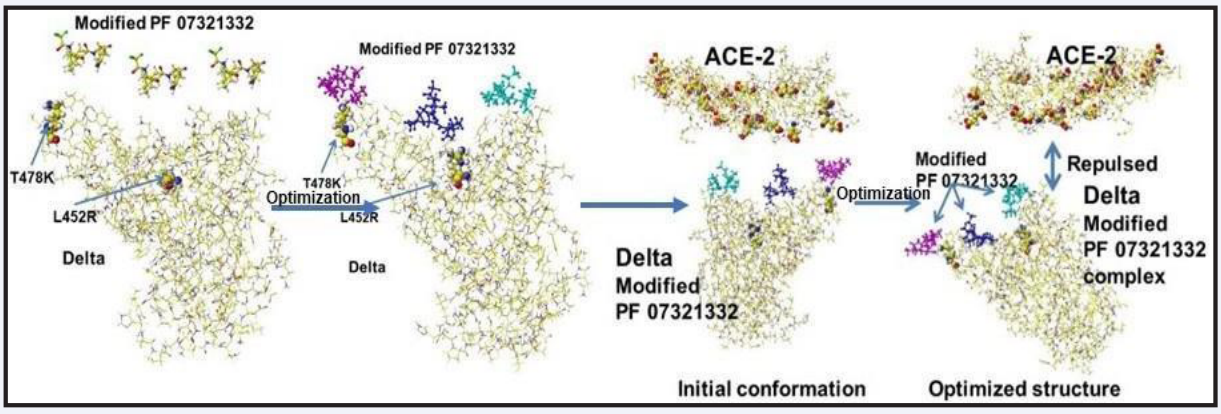


Figure 6 Initial conformations of Delta S-RBD with three modified PF 07321332 molecules, the complex (optimized) structure of Delta S-RBD with three modified PF 07321332 molecules complex, and ACE-2 and their optimized structure.

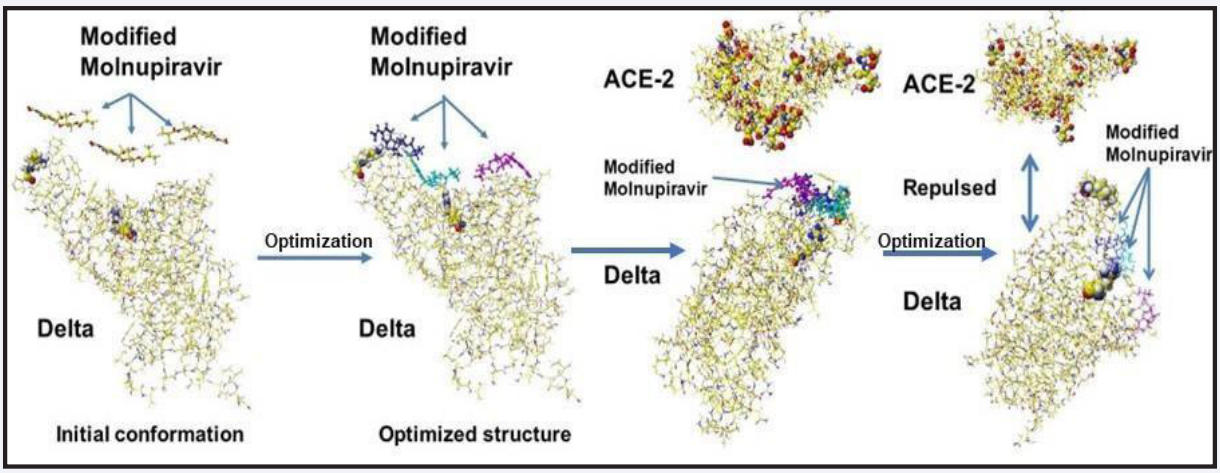


Figure 7 Initial conformations of Delta S-RBD with three modified Molnupiravir molecules, the complex (optimized) structure of Delta S-RBD with three modified Molnupiravir molecules complex and ACE-2 and their optimized structure.

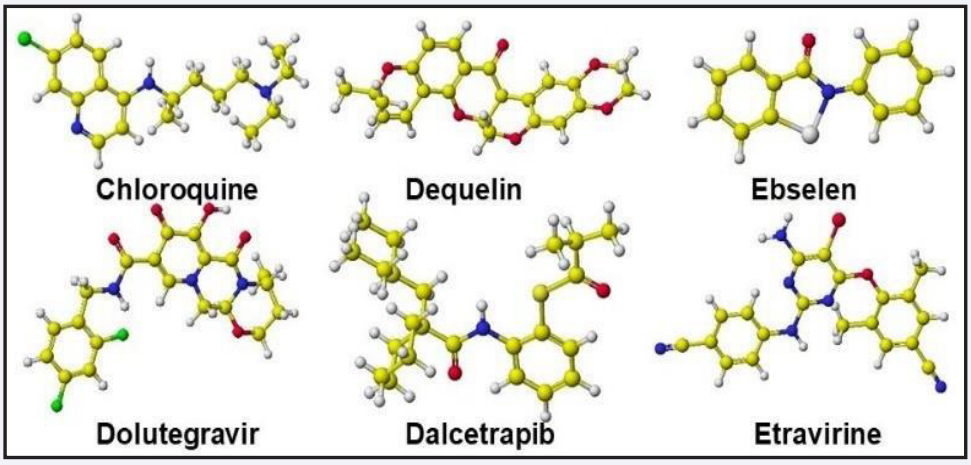


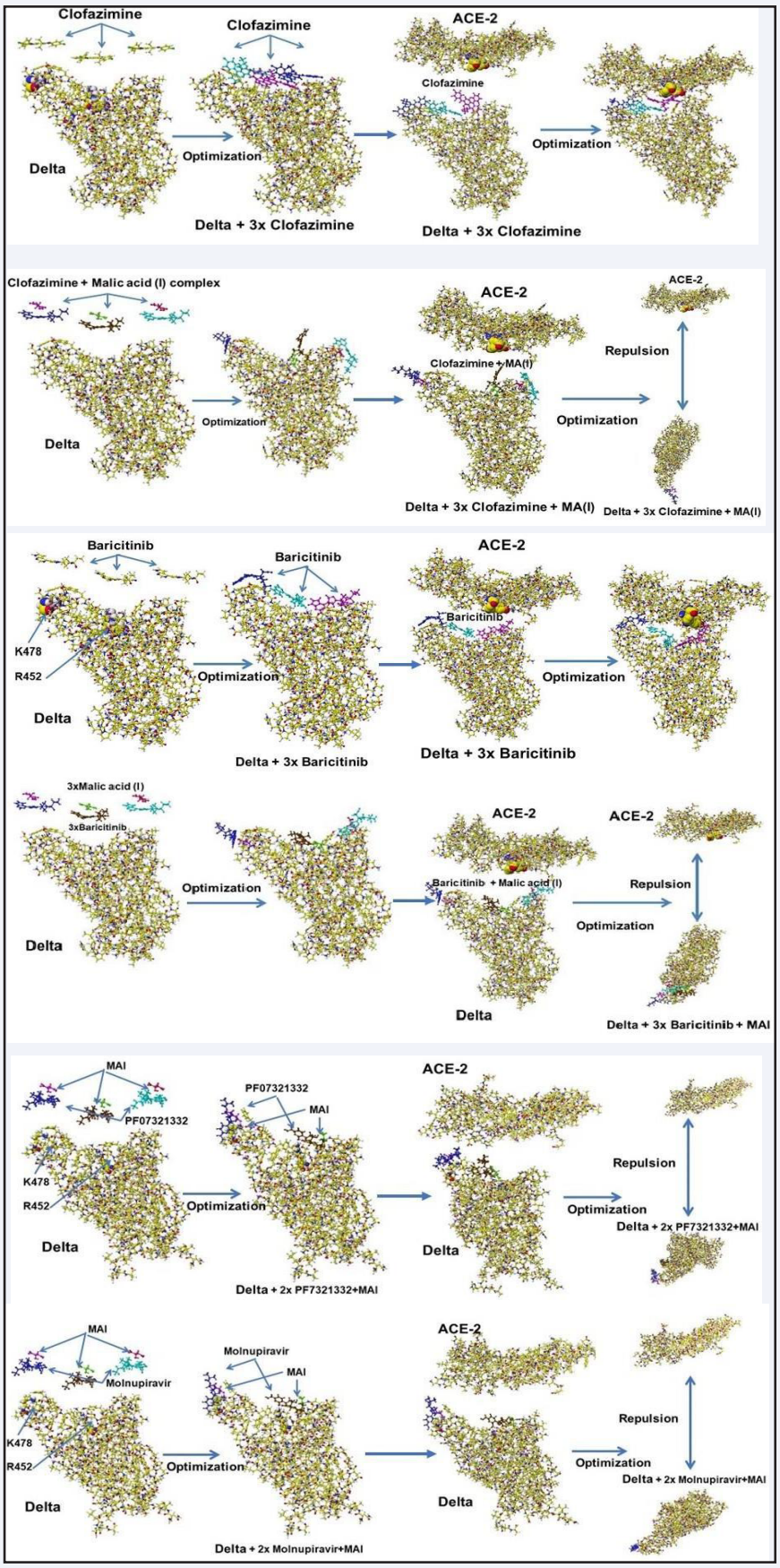
Figure 8 Structure of Chloroquine, Dalcetrapib, Deguetin, Dolutegravir, Ebselen, and Etravine, where atoms are indicated as color or grayscale; nitrogen (blue or dark gray balls), oxygen (red or black balls), carbon (yellow or gray balls), hydrogen (small white balls), and fluorine (light green of light gray balls). Generally, fluorone atoms support the sterically stable structure.

The binding sites of PF07321332 and Molnupiravir are known, but these six compounds have no similarity to replace these known compounds. Ensitrelvir has two basic groups that may bind two phosphates of DNA and RNA, like derivatized β-alanine, which was once an excellent target compound in medicines. These six compounds may not have the capability of PF07321332 and Monupiravir. TCA-cycle acids can inhibit the binding of the variant and ACE-2, and medicine is required to inhibit the multiplication of the protein. If these six compounds inhibit the multiplication of COVID-19 protein, the cocktail with organic acid may improve medical activity.

These compounds did not inhibit the binding of BA.2.86 S-RBD binding with ACE-2. Considering the covering of basic amino acids, organic acid should face the ACE-2 binding site. Their malic acid complexes were prepared for their cocktail dose, and their malic acid complexes bound S-RBD were faced with an ACE-2 binding site, then optimized their conformations. The optimized structures of these six compounds are shown in Figure 9. These visualized Figures clearly exhibit the useful dose of cocktail medicines.

A possible approach to improve the known medicine-activity

Xocoba (Ensitrelvir Fumaric Acid) is like an extended β-alanine derivative and may bind RNA and/or DNA phosphate and inhibit the multiplication. Xocoba was later modified as fumarate and improved the drug activity (Ensitrelvir Fumaric Acid). It is a very basic compound and easily binds to the ACE-2 binding site via ion-ion interaction. Furthermore, replacing a group with a carboxy group like PF07321332 is difficult. The basic property easily makes a complex with carboxyl acid.


Clofazimine
Clofazimine
ACE-2
Clofazimine
Delta
Optimization
Optimization
Delta + 3x Clofazimine
Delta + 3x Clofazimine
Clofazimine + Malic acid (I) complex
ACE-2
ACE-2
Clofazimine + MA(I)
Repulsion
Delta
Optimization
Optimization
Delta + 3x Clofazimine + MA(I)
Delta + 3x Clofazimine + MA(I)
Baricitinib
Baricitinib
ACE-2
Baricitinib
K478
R452
Optimization
Optimization
Delta
Delta + 3x Baricitinib
Delta + 3x Baricitinib
3xMalic acid (I)
3xBaricitinib
ACE-2
ACE-2
Optimization
Baricitinib + Malic acid (I)
Repulsion
Optimization
Delta
Delta
Delta + 3x Baricitinib + MAI
MAI
PF07321332
ACE-2
PF07321332
MAI
Repulsion
K478
R452
Optimization
Optimization
Delta + 2x PF7321332+MAI
Delta
Delta
Delta + 2x PF7321332+MAI
MAI
Molnupiravir
ACE-2
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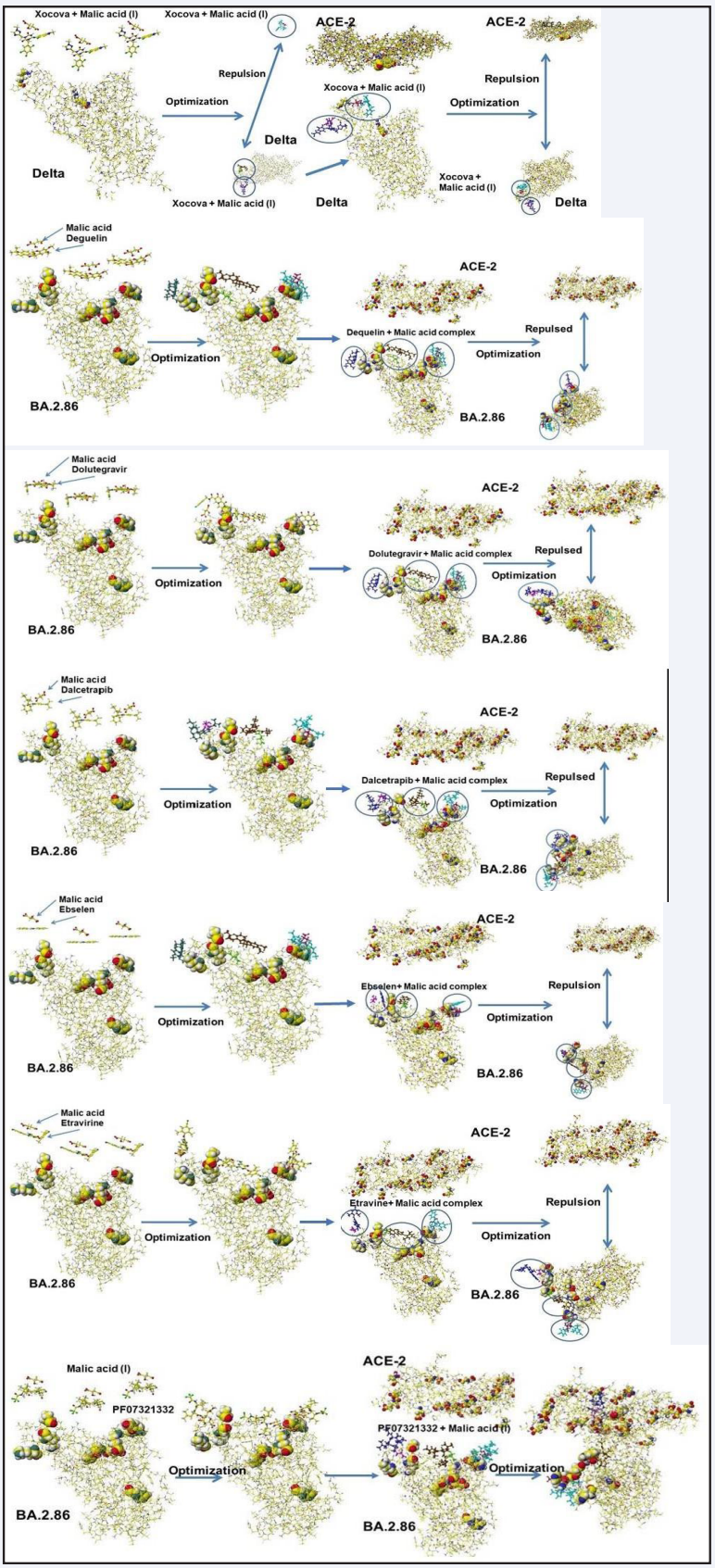

Figure 9 Optimized bound structures of Chloroquine, Dalcetrapib, Deguetin, Dolutegravir, Ebselen, and Etravine..

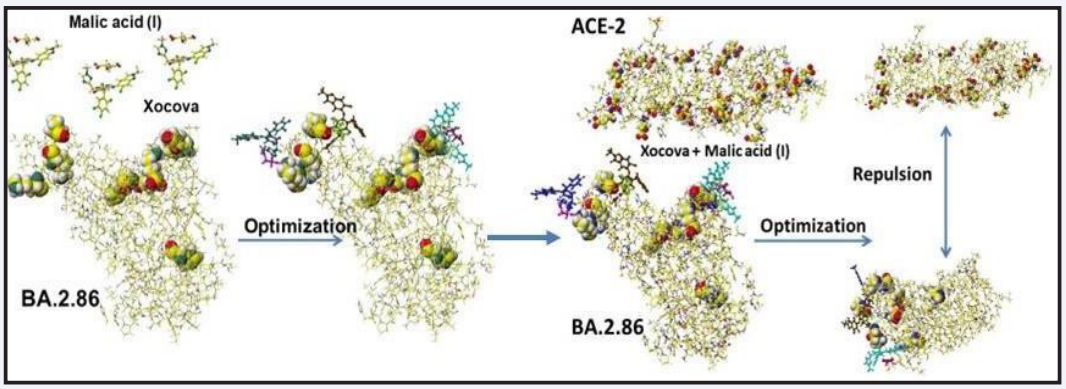

Figure 10 Effect of cocktail medicines with malic acid inhibited BA.2.86 binding with ACE-2.

The Xocova bound Omicron BA.2 S-RBD The complex faces ACE-2 and optimized the structure. The optimized structure exhibits that Xocova did not inhibit the binding. The effective use of Xocova may bind mRNA and inhibit the multiplication but requires the modification of cocktail use. Therefore, the feasibility of the cocktail with malic acid was studied. The cocktail performance of PF07321332 and Molnupiravir is also exhibited in Figure 10. However, the PF07321332 and malic acid cocktail was not powerful enough to inhibit the binding.

Various compounds have been applied for Covid-19 treatment. Acidic compounds of cannabinoids did block the cellular entry of SARS-CoV-2 [22]. Pros and Cons results have been reported for Ivermectin because the saccharides adsorb glycoproteins and damage their activity [23,24]. Effectiveness of several compounds such as Ursodeoxycholic acid [25], a combination of Nirmatrelvir and Ritonavir [26], fungal metabolites [27], Bruceine A [28], Gamabufotalin [28], VITAMIC BIOSEN [29], prophylactics [30], and Azvudine [31] was reported and Sotrovimab remained weakly reactive and broadly neutralizing antibody SA55 was still highly effective [32]. However, Nirmatrelvir, Ritonavir, Bruceine A and D, Gamabufotalin, and Azvudine were adsorbed on the ACE-2 in the above experiment. Ursodeoxycholic acid was repulsed from the ACE-2. Further study is required for their practical use.

Omicron JN.1 is further mutated valiant from Omicron XBB.1.5 and exhibited stronger binding affinity with ACE-2 than that of BA.2.86. The JN.1 mutation of amino acid from leucine to serine (L455S) reduced the steric hindrance for the S-RBD binding with ACE-2 and enhanced the binding affinity [33,34]. The mutation L455S increases the flexibility of JN.1 R454. The mutation contributed to a strong binding affinity of JN.1 [35]. Therefore, further extended study for JN.1 is necessary. Sweden experienced fewer deaths per population unit during pandemic seasons than most high-income countries and was comparable to neighboring Nordic countries [35], and the modified vaccine virus Anlara vector expressing the SARS-CoV-2 spike protein conferred full protection against SARS-CoV-2 cerebral infection may be a promising vaccine candidate against SARS-CoV-2 [36]; therefore, we should study our immune system capability and further practical use of current practice medicines.

CONCLUSIONS

There are two types of drugs. One is trapped by proteins (enzymes) and is not metabolized by the protein. This type of compound is a suicide helper, and various acidic drugs have been developed against enzyme activity. Basic compounds (drugs) bind with the phosphate of DNA and RNA and inhibit the multiplication, but they work without specificity. Molnupiravir and Xocova have basic groups for binding with the phosphate of DNA and RNA; however, they do not inhibit the S-RBD and ACE-2 binding. PF07321332 was also trapped by ACE-2. If these compounds are not trapped by ACE-2 and keep ACE-2 enzyme activities, they may work as active drugs. The modification is one solution, and a cocktail with TCA acids is another solution. In physiological conditions, ionized carboxy and phenolic hydroxy groups can inhibit the S-RBS and ACE-2 binding. Therefore, a simple solution to add inhibition capability is a cocktail dose using known medicine with TCA acids. It does not take time to apply a mixture for the treatment because TCA acids are not specific medicines and are contained in everyday foods. Even. We produce them in our metabolic pathways; kids primarily produce them a lot. Such cocktail drugs consisting of a mixture of the drug candidate and acidic compounds may help the feasibility of the proposed compounds.

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