

Review Article

Paxlovid: Mechanism of Action, Synthesis, and *In Silico* Study

Mahrokh Marzi,¹ Mohammad Kazem Vakil,² Maryam Bahmanyar,¹
and Elham Zarenezhad ¹

¹Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran

²Department of Internal Medicine, School of Medicine, Fasa University of Medical Science, Fasa, Iran

Correspondence should be addressed to Elham Zarenezhad; el.zarenezhad@gmail.com

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In this work, the discovery and description of PF-07321332, a major bioavailable oral SARS-CoV-2 protease inhibitor with in vitro human coronavirus antiviral activity, and excellent selection of off-target and in vivo immune profiles are reported. Various drugs and novel compound candidates for the treatment of the COVID-19 pandemic have been developed. PF-07321332 (or nirmatrelvir) is a new oral antiviral drug developed by Pfizer. In response to the pandemic, Pfizer has developed the COVID vaccine and in 2022 will launch its new major anti-SARS-Cov-2 protease inhibitor (PI). The combination of ritonavir and nirmatrelvir is under study in phase III of the clinical trial with a brand name Paxlovid. Paxlovid is an active 3Cl protease inhibitor. Paxlovid exerts its antiviral efficacy by inhibiting a necessary protease in the viral replication procedure. Proteases of coronavirus cleave several sites in the viral polyprotein where pyrrolidone was replaced by flexible glutamine. Due to the coronavirus pandemic, there is high demand for synthesis and development of this novel drug. Herein, we report the synthetic route and the mechanism of action was recently published on nirmatrelvir. Also, a comparison of the performance of two new oral antiviruses (molnupiravir and nirmatrelvir) for the treatment of COVID-19 is described. This review will be helpful for different disciplines such as biochemistry, organic chemistry, medicinal chemistry, and pharmacology.

1. Introduction

Since the 19th century, one of the great threats to public health and safety is the COVID-19 pandemic that resulted from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2]. Until December 12, 2021, over 269 million cases and 5.3 million deaths have been demonstrated, making the COVID-19 pandemic [3]. Nirmatrelvir is a novel antiviral drug developed by Pfizer [4]. It blocks the activity of an enzyme that the virus needs to replicate. This analog of GC373 is an orally active 3Cl protease inhibitor. Corona viral proteases split multiple situations, generally after glutamine. Researchers demonstrated that the glutamine could be displaced by a hard pyrrolidone [5, 6]. 2-Pyrrolidone is a colorless liquid that can be mixed with water and other organic solvents [7]. 2-Pyrrolidone and different derivatives produced from it have various industrial

uses. Nirmatrelvir acts as a covalent inhibitor that binds directly to the catalytic cysteine (Cys145) residue [8]. A phase II/III clinical trial has shown a promising drug resulting in an 89% reduction in hospitalizations, when patient is given within three days after symptom onset [9]. Recently, on November 16, 2021, the results of the phase 2/3 trial of Pfizer were presented to the U.S. Food and Drug Administration (FDA) for emergency authorization. The modification of lufotrelvir as a human clinical antiviral candidate, PF-07321332 (or nirmatrelvir), was developed [10]. Lufotrelvir is a covalent inhibitor in which the warhead is a phosphate prodrug of hydroxyketone [11]. Nirmatrelvir resulted by some modification of tripeptide protein mimetic of lufotrelvir that makes it useful for oral administration [4]. The oral form of nirmatrelvir/ritonavir offers the most promising therapeutic effect compared to these novel medicines (89% reduced risk of hospitalization or death) [12–16]

(Figure 1). Nirmatrelvir/ritonavir is expected to reverse the COVID-19 pandemic [17–19].

This review is focused on the general synthetic pathway, the mechanism of action, and molecular docking study to provide an insight for the logical synthesis of more effective nirmatrelvir as an orally antiviral candidate. The diagram of choosing publications and the content of this review is illustrated in Figure 2.

2. PF-07321332 (or Nirmatrelvir) Development Strategies against SARS-CoV-2

From the beginning, the Pfizer Company has created a modern, useful oral antiviral for SARS-CoV-2 that behaves by controlling the infection that causes COVID-19. Nirmatrelvir, as the recent molecule (the primary protease inhibitor of SARS-CoV-2), has revealed its ability by inhibiting the replication activity of the SARS-CoV-2 virus *in vitro* and by cysteine residues, which acted as a reversible covalent inhibitor of the major protease of SARS-CoV-2. This drug is perceived as a possible treatment for future coronavirus risks. Nirmatrelvir is prescribed at the first symptom, and patients do not require to be hospitalized. This new antiviral drug is in phase one clinical trial. PF-07304814 is another compound reported by Pfizer Company with intravenous injection for hospitalized victims, and this drug is located in phase 1b multidose trial now. These antiviral drugs are being developed, as novel chemical organisms (*de novo* compounds) (Figure 3) [20, 21].

3. Synthesis of PF-07321332 (or Nirmatrelvir)

For the first time, Pfizer scientists published the complete particularities of the synthesis of nirmatrelvir [4]. In step (1), the water-soluble carbodiimide EDCI (as a coupling factor) paired homochiral amino amide with a synthetic homochiral amino acid. The resulting intermediate is then treated with Burgess reagent, which dehydrates the amide group to the nitrile of the product. This compound binds instantly to the catalytic cysteine (Cys145) residue of the cysteine protease enzyme (as a covalent inhibitor) (Scheme 1) [8].

3.1. Synthesis of *N*-[(2*S*)-1-((1*S*)-1-Cyano-2-[(3*S*)-2-Oxopyrrolidin-3-yl]Ethyl)Amino]-4-Methyl-1-Oxopentan-2-yl]-4-Methoxy-1*H*-Indole-2-Carboxamide (6). Compound (1) [22] was stirred in a solution of hydrogen chloride in ethyl acetate and methanol for 1 h at 25°C. *O*-(7-azabenzotriazol-1-yl)-*N*, *N*, *N*′, *N*′-tetramethyluronium hexafluorophosphate (HATU) and *N*, *N*-diisopropylethylamine were added to 0°C solution of compound (2) and 4-methoxy-1*H*-indole-2-carboxylic acid in *N*, *N*-dimethylformamide. The solution was extracted after stirring at 0°C for 1.5 hours and pouring in water/ice with ethyl acetate. These organic layers with saturated aqueous sodium chloride (NaCl) solution were washed and dried by sodium sulfate [23]. Chromatography of silica gel (eluent: 1:10 methanol/dichloromethane) presented (4) as a yellow oil. The following was added a solution of ammonia in methanol to a resolu- tion (4) in methanol. Following stirring, the reaction

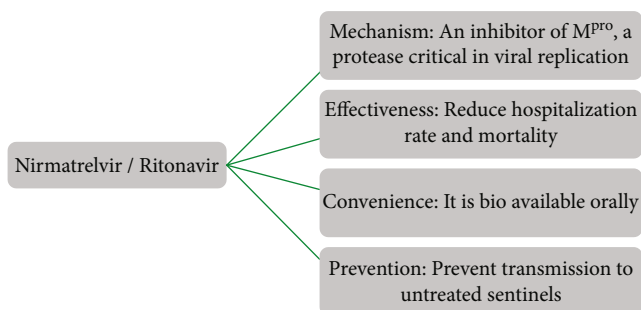


FIGURE 1: Introducing the oral form of nirmatrelvir/ritonavir as having the most promising therapeutic effect among new drugs [12].

combination for 6 hours at room temperature was again added ammonia solution in methanol and stirring was continued during the night. After readding ammonia solution to methanol and stirring overnight, the last treatment was performed with ammonia solution in methanol. The reaction mixture was concentrated *in vacuo* after stirring for another day. Compound (5) was obtained after mixing the residue with the product of the same reaction performed by (4) and repeatedly dissolving the reaction mixture in ethyl acetate and concentrating. In a mixture of dichloromethane and pyridine, 1*H*-imidazole and solution (5) were cooled to −35°C (by an acetonitrile/dry ice bath). Then, phosphorus oxychloride was added over 5 minutes in a dropwise manner. The reaction was stirred for about 1.5 h (between −30°C and −20°C), then acted with HCl (hydrochloric acid), and stirred for 1 h. The resulting organic layers were concentrated *in vacuo* (after drying over sodium sulfate and filtering). The residue was blended with purified (6) from a distinctive class to produce (6) as a solid (subjected to silica gel chromatography ethyl acetate in methanol). This compound was combined with a similar reaction product performed by (5); then, it was stirred and was filtered. Compound (6) was obtained as a solid after washing with heptane and diethyl ether (Scheme 2) [4].

3.2. Synthesis of (1*R*,2*S*,5*S*)-*N*-{(2*S*)-1-(1,3-Benzothiazol-2-yl)-1-Oxo-3-[(3*S*)-2-Oxopyrrolidin-3-yl]Propan-2-yl}-3-[(4-Methoxy-1*H*-Indol-2-yl)Carbonyl]-6,6-Dimethyl-3-Azabicyclo [3.1.0] Hexane-2-Carboxamide (13). Trifluoroacetic acid (TFA) was added to a 0°C solution of (7) [24] in dichloromethane (CH₂Cl₂). This mixture was stirred for 1 hour at 0°C. TFA salt of the amine (8) was cooled to 0°C and treated with *N*, *N*-diisopropylethylamine. In a different vial, a mixture of compound (9), a drop of DIES (*N*, *N*-diisopropylethylamine) in DMF (*N*, *N*-dimethylformamide), and HATU was stirred until a solution was earned (at room temperature). After adding this solution to the amine salt solution, the reaction mixture was heated at room temperature for 24 h. Compound (10) was obtained as a clear yellow oil after washing, drying, filtering, and concentrating. Then, TFA was added to a 0°C solution of (10) in CH₂Cl₂. Compound (11) was obtained after concentrating, cooling to 0°C, dissolving in DMF, and treating with DIEA. A solution of (12), a drop of DIEA in DMF, and HATU was added to this mixture. After

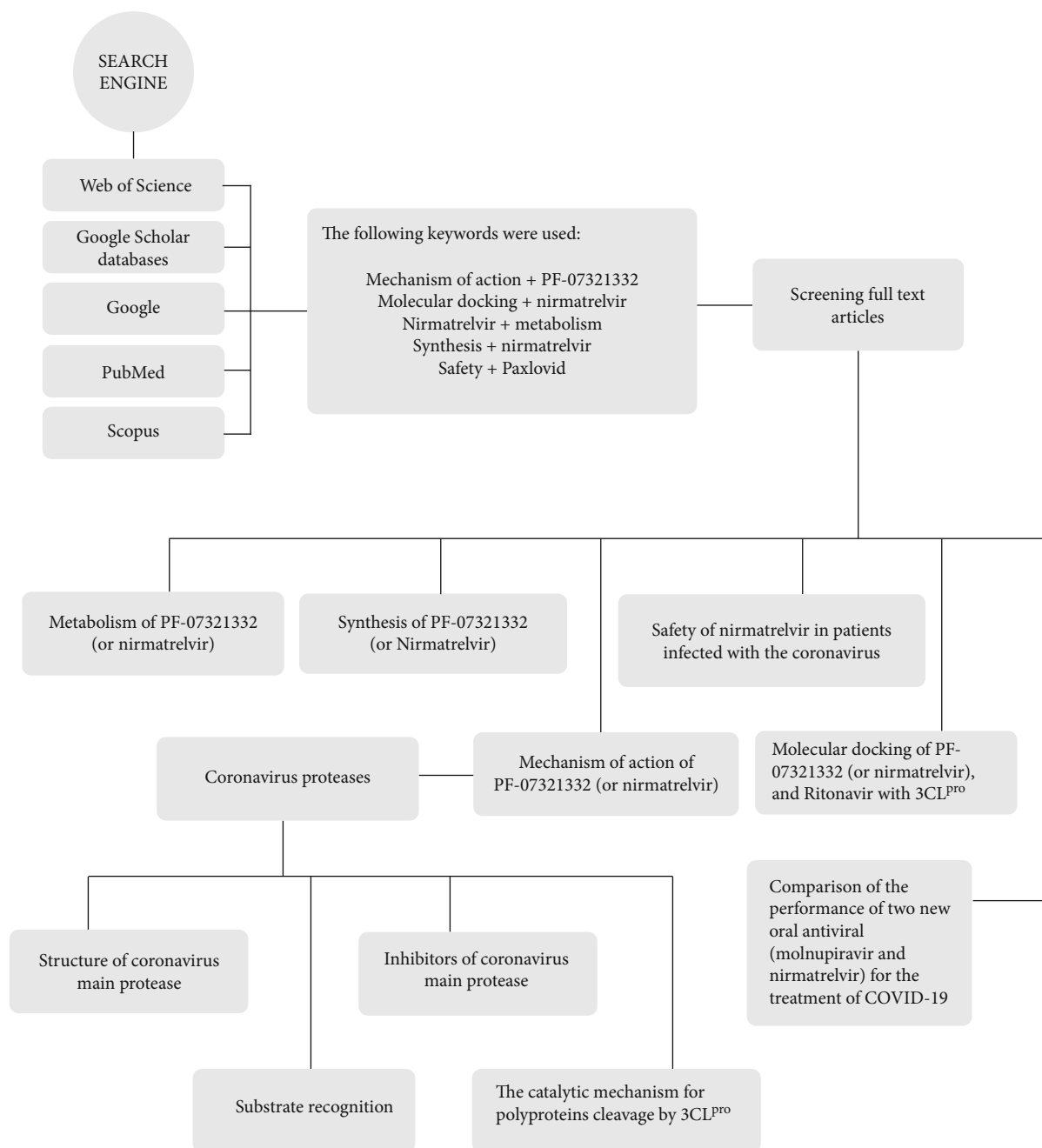


FIGURE 2: Chart related to the content provided in this review.

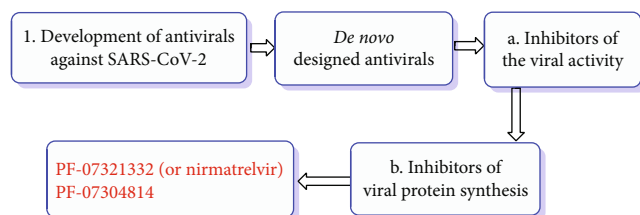
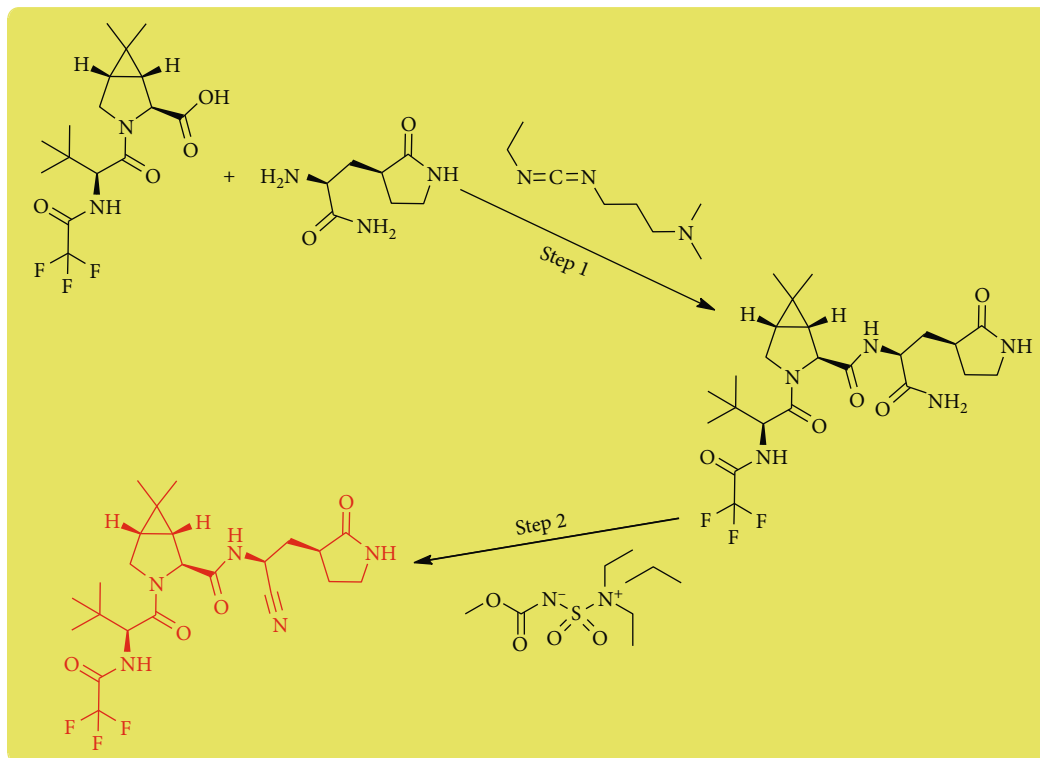


FIGURE 3: Antiviral drug development strategies against SARS-CoV-2.

2 hours, the reaction combination was diluted with EtOAc to form compound (13) (Scheme 3) [4].

3.3. *Synthesis of (1R,2S,5S)-N-((2S)-1-(1,3-Benzothiazol-2-yl)-1-Oxo-3-[(3S)-2-Oxopyrrolidin-3-yl]Propan-2-yl)-6,6-Dimethyl-3-[N-(Methylsulfonyl)-L-Valyl]-3-Azabicyclo [3.1.0] Hexane-2 Carboxamide (19)*. Solution (7) in CH_2Cl_2 was treated with a solution of hydrochloride in 1,4-dioxane and then using ethyl acetate. The reaction mixture was stirred at room temperature, and then, it was condensed *in vacuo*. The remaining trituration with diethyl ether (Et_2O) obtained a compound of bright-yellow solid (the HCl salt of (8)) that



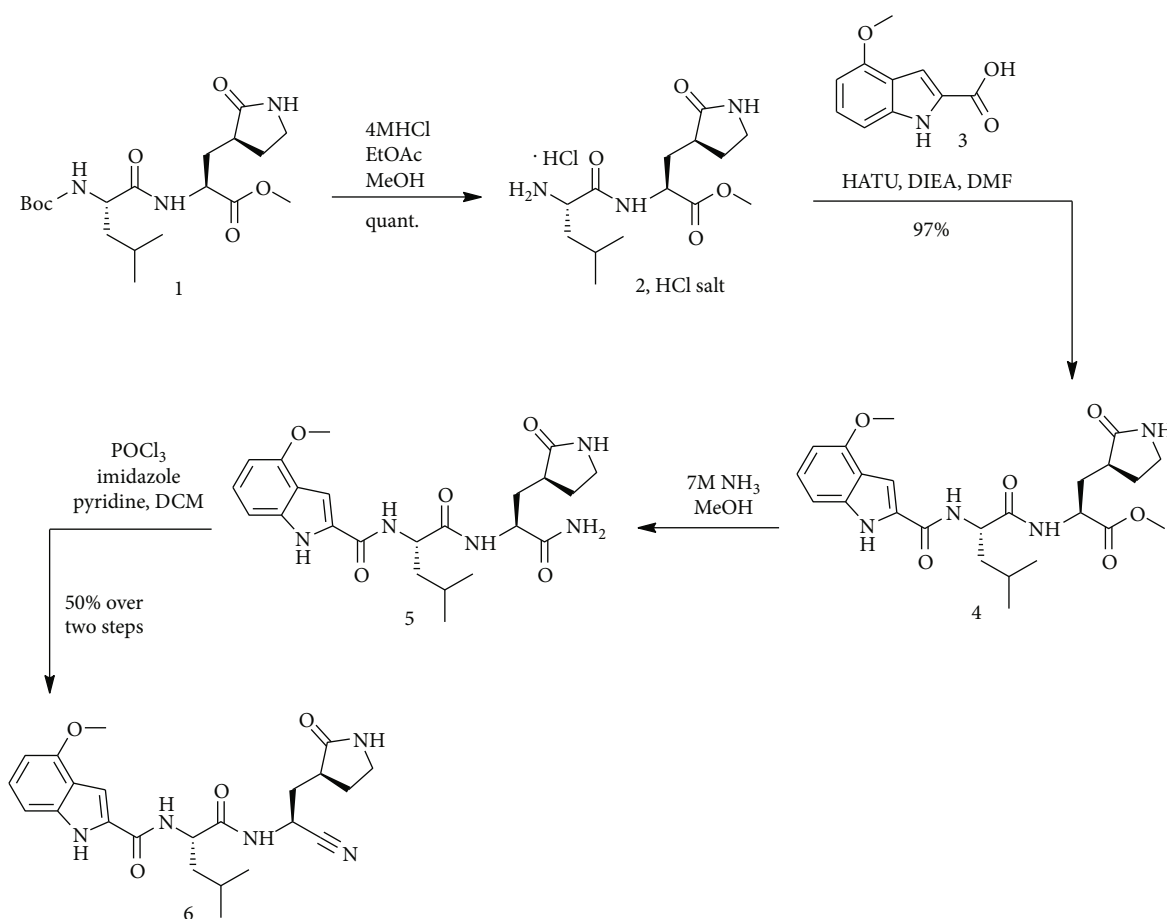
SCHEME 1: A common pathway for synthesis of PF-07321332 (or nirmatrelvir).

was utilized without more purification in the next step. In the following, a 0°C mixture of (14), (15), and DMF was treated with HATU. Then, stirring was continued after the addition of DIEA (for 2 h at 0°C). The incorporated organic layers were concentrated *in vacuo* and were prepared (16) as gum. Aqueous LiOH (lithium hydroxide solution) was poured in the form dropwise into a 0°C solution of (16) in a mixture of methanol (CH₃OH) and tetrahydrofuran. Aqueous LiOH solution was added to the stirred reaction mixture for 2 h at 0°C and 4 h at room temperature, and then, stirring was continued for 15 minutes. After repeating the previous operation, compound (17) was obtained as an off-white solid. To a 0°C solution of (17), HATU and N,N-diisopropylethylamine in N,N-dimethylformamide were added (8), hydrochloride salt. DIEA was added, and the reaction mixture was stirred and made warm to room temperature overnight. Compound (18) was provided as oil after dilution by ethyl acetate. To a solution of compound (18) in CH₂Cl₂, a solution of HCl in 1,4-dioxane was added and the reaction mixture was stirred for 2 h at room temperature. Stirring was continued, and then, the reaction mixture was concentrated. To this mixture, triethylamine (Et₃N) and methanesulfonyl chloride were added. Then, compound (19) was produced as a white solid (Scheme 4) [4].

3.4. Synthesis of (1R,2S,5S)-N-((2S)-1-(1,3-Benzothiazol-2-yl)-1-Oxo-3-[(3S)-2-Oxopyrrolidin-3-yl]Propan-2-yl)-6,6-Dimethyl-3-[N-(Trifluoroacetyl)-L-Valyl]-3-Azabicyclo [3.1.0] Hexane-2-Carboxamide (23). A solution of compound (7) in hexafluoroisopropanol (HFIP) was cooled in an ice bath and was treated with para-toluenesulfonic acid. After the reaction

mixture had been stirred at room temperature it was concentrated; trituration of the residue with ethyl acetate provided (8) para-toluenesulfonate salt. Then, a solution of HCl in 1,4-dioxane is added to the solution (17) in CH₂Cl₂ and mixed. A white solid (20) was obtained by removing the solvent. To a 0°C solution of (20) in CH₃OH, Et₃N was added, and then, the reaction mixture was warmed. After adding compound (21) to the reaction mixture, compound (22) was produced as a white solid. In the next step, EDCI was added to combination (8), para-toluenesulfonate salt, and a solution of (22) in anhydrous acetonitrile at 0°C. Pyridine was added dropwise to the stirring reaction mixture. Filtration acquired (23) as a white solid (Scheme 5) [4].

3.5. Synthesis of PF-07321332 or Nirmatrelvir (33). Compound (25) was obtained as a yellow solid by adding ammonia solution in methanol to compound (24). The solution of HCl in isopropanol was added to a 0°C solution of (25) in isopropanol. Then, it was concentrated to give the HCl salt of (26). OHATU was added to a 0°C solution of (15) and (27) in a mixture of DMF and acetonitrile (CH₃CN), followed by dropwise addition of DIEA. Then, compound (28) as a colorless oil was provided. To a solution of (28) in tetrahydrofuran, water and lithium hydroxide (LiOH) were added. Compound (29) was produced after washing, drying, and concentrating. To a solution of (29) in CH₂Cl₂, a solution of hydrochloride in 1,4-dioxane was added and the reaction mixture was stirred for 16 h at room temperature. By removing the solvents, HCl salt of (30) was generated. To a solution of the HCl salt of (30) in CH₃OH, Et₃N was added, followed by compound (21); then, the reaction



SCHEME 2: Synthesis path of compound (6).

mixture was warmed to 50°C and was stirred. The incorporated organic layers were washed with sodium chloride (NaCl) solution, and compound (31) was obtained as a white solid. To a solution of (31) and the HCl salt of (26) in butan-2-one, 2-hydroxypyridine 1-oxide was added and the mixture reaction was cooled (to 0°C). Then, DIEA and 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride (EDCI) were added and compound (32) was provided. For preparing compound (33) (MTBE solvate), a solution of (32) in CH₂Cl₂ to methyl *N*-(triethylammoniosulfonyl) carbamate and inner salt (Burgess reagent) was added. Compound (33) (anhydrous MTBE solvate) was charged to a reactor at 350 rpm. Stirring was continued overnight with the addition of heptane and isopropyl acetate (IPAC). Compound (33) was acquired as a white crystalline solid (Scheme 6) [4].

During the discussion of synthesis methods, different reaction parameters (temperature, solvent, etc.) affected selectivity and efficiency (Table 1) [14].

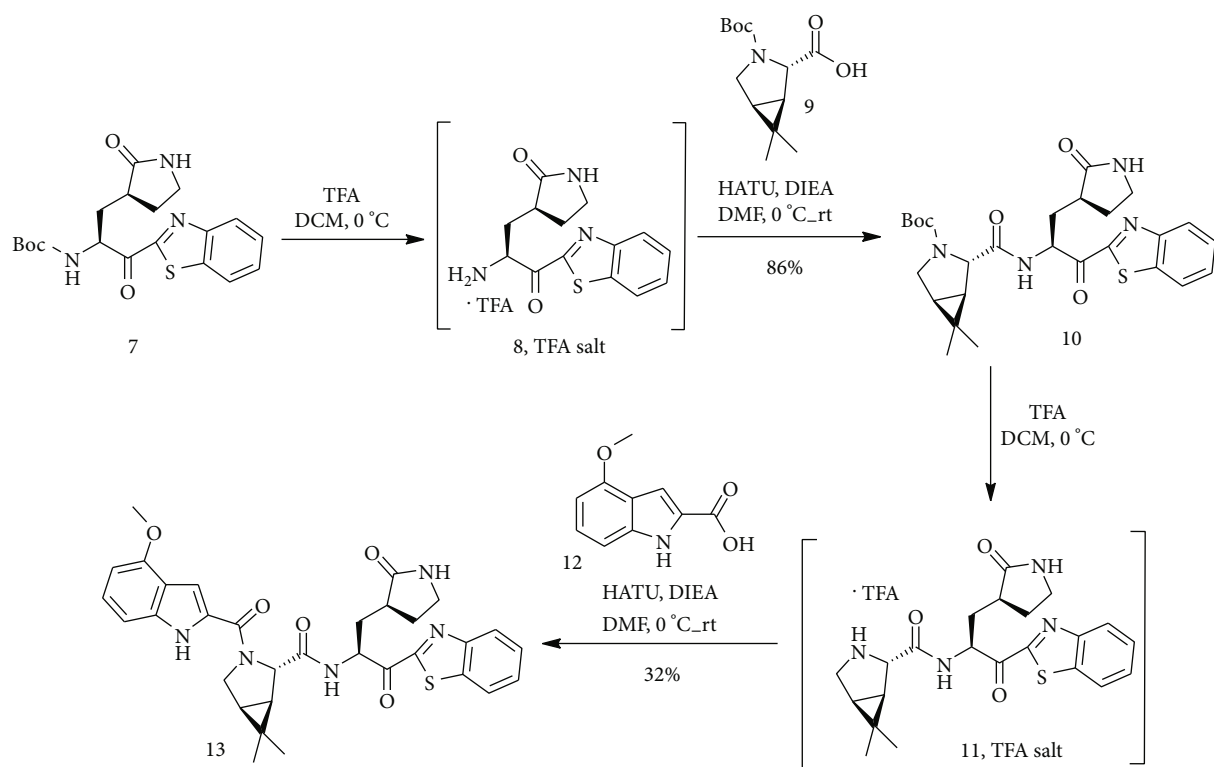
4. Mechanism of Action of PF-07321332 (or Nirmatrelvir)

For the treatment of COVID-19, the antiviral medicine of nirmatrelvir reacts as an orally active inhibitor of 3CL protease. The blend of ritonavir with nirmatrelvir is in phase III

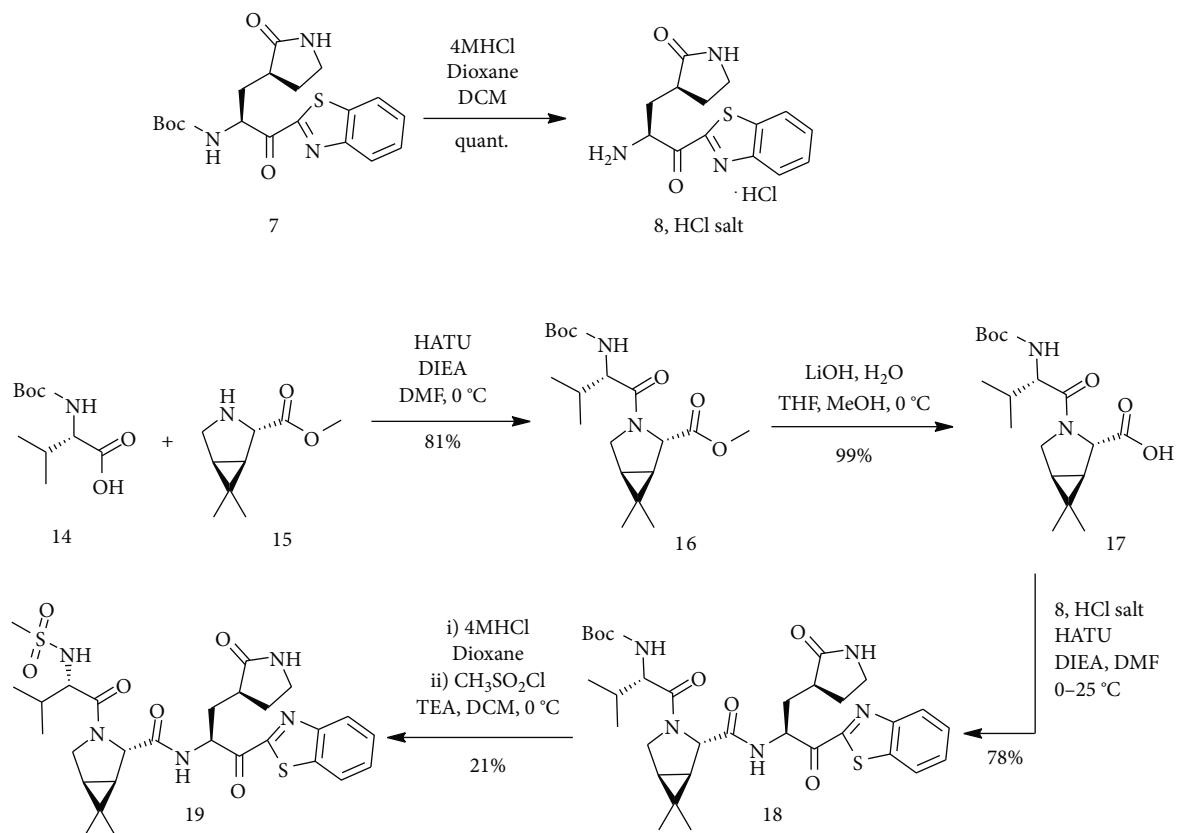
trials [25–29], and under the brand name, Paxlovid is anticipated to be sold [30]. By cytochrome enzymes, ritonavir dwindles the metabolism of nirmatrelvir to preserve premier concentrations of the principal drug [31].

4.1. Coronavirus Proteases. The severe acute respiratory syndrome coronavirus 2 is the cause of the coronavirus epidemic [32]. By mid-2021, more than 170 million confirmed infections had been announced worldwide. In the 21st century, the 2019 epidemic is the third outbreak of coronavirus [33, 34]. Different types of the syndrome such as the severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome (MERS-CoV), and SARS-CoV-2 depend on the *Betacoronavirus* genus (the *Coronaviridae* family). Coronaviruses encoded papain-like protease (PL^{Pro}) (also known as nsp3) and main protease (M^{Pro}) (also known as 3CL^{Pro} or nsp5) to split the viral polyprotein [35]. M^{Pro} has a notable role in polyprotein processing as a more tractable therapeutic objective (Figures 4(a) and 4(b)) [36]. M^{Pro} from SARS-CoV-1 and SARS-CoV-2 has an excellent general sequence identity [36, 37].

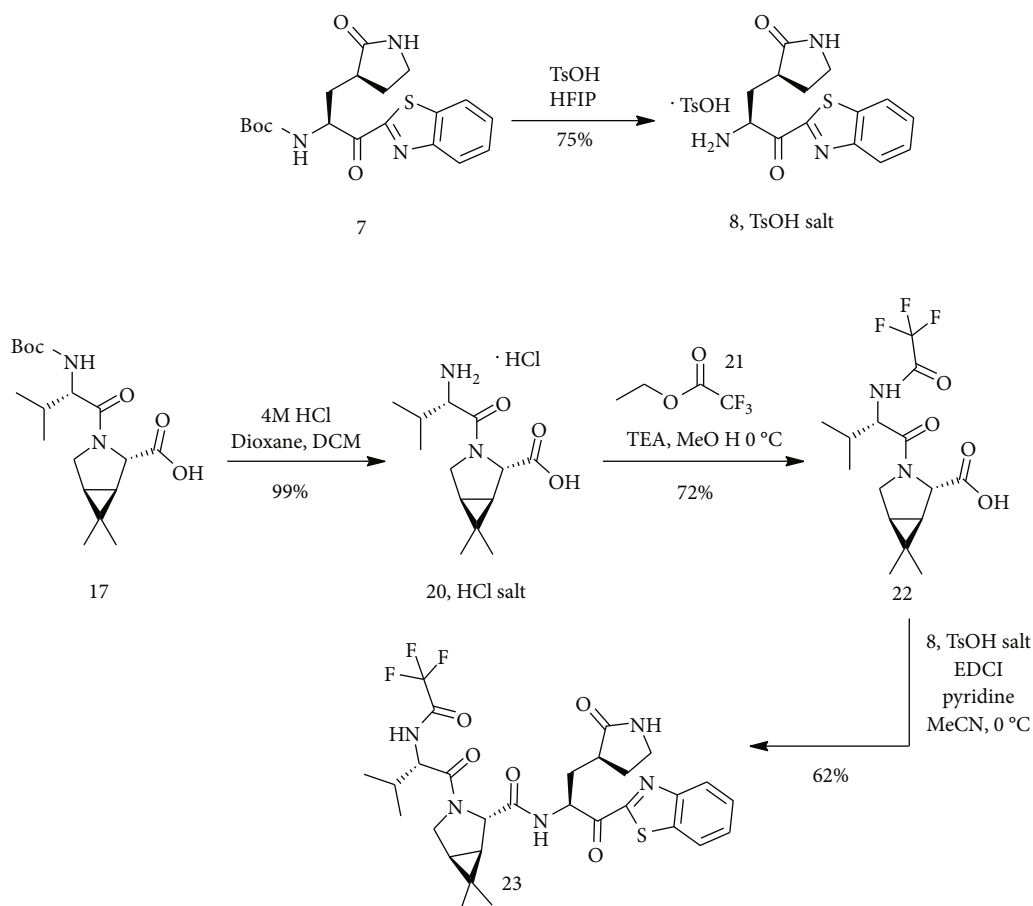
4.1.1. Structure of the Coronavirus Main Protease. M^{Pro} is a residual protease of 306 with a catalytic binary consisting of histidine and cysteine. Active-state M^{Pro} is a dimer, in which the two monomers are arranged orthogonally



SCHEME 3: Synthesis path of compound (13).



SCHEME 4: Synthesis path of compound (19).



SCHEME 5: Synthesis path of compound (23).

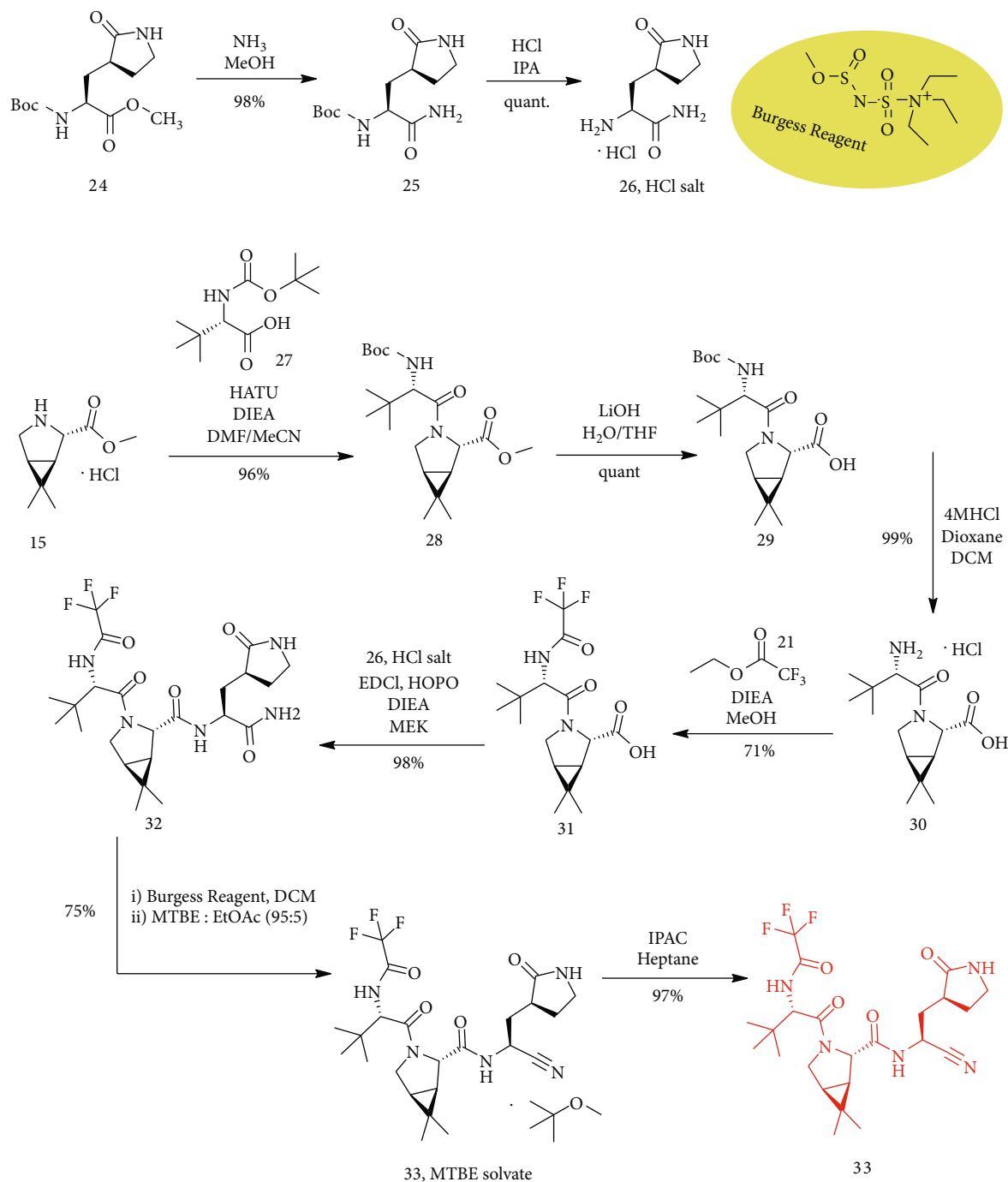
(biologically) [38]. Each monomer consists of three areas; two anti- β barrels parallel to the fold-like trypsin-like serine proteases are built by areas I and areas II together. Area III includes five α -helices which are attached by the linker to area II. Dimerization of the protomers occurs at the N-terminus (N-finger) located between domains II and III [39, 40].

4.1.2. Substrate Recognition. In addition to the catalytic binary consisting of H47 and C145, M^{pro} contains a molecule of water buried in the active site that replaces the aspartate residue, which is usually combined with histidine and cysteine in the conventional catalytic triplet of other proteases [36, 41]. A multistep mechanism pursues the catalytic process, in which H47 removes the catalytic cysteine from the proton and produces a nucleophilic thiol that attacks the carbonyl carbon of the scissor bond and forms a quadrilateral intermediate. The intermediate falls and remains an acylated intermediate. The thiol side chain is regenerated by histidine, which activates a water molecule and hydrolyzes the intermediate [42, 43]. As shown in Figure 2, M^{pro} at 11 sites splits the viral polyproteins (pp1a and pp1ab). The amino acid sequence is varied at the sites of cleavage, except in P1, which is always glutamine, and P2, which is preferred for leucine. The P3 situation can insert hydrophilic

and hydrophobic residues, and for amino acids with tiny side chains, P4 has superiority. M^{pro} identifies substrates spanning P4-P1 [44].

4.1.3. Inhibitors of the Coronavirus Main Protease. Inhibitors of $-2 M^{pro}$ and SARS-CoV-1 have been evaluated widely [37, 42, 45, 46]. Small molecules and peptidomimetics containing Michael receptors, for example, ketones and aldehydes, form a significant part of the designed inhibitors. The P1 γ -lactam group as a glutamine mimetic is a standard feature of these inhibitors. PF-07321332 (or nirmatrelvir) and PF-07304814 (two dipeptidyl inhibitors) are arriving phase I clinical trials (NCT04756531 and NCT04535167) [10, 11]. PF-07304814, the prodrug form of PF-00835231, contains a phosphonate group for better solubility that gets cleaved by alkaline phosphatase enzymes in tissue. Nirmatrelvir was supposed to dominate the intravenous delivery method for PF-07304814.

4.1.4. The Catalytic Mechanism for Polyprotein Cleavage by 3CL pro . By inferring the structure and analysis of the SARS-CoV 3CL pro [42] autolytic section (automated processing), a universal nucleophilic reaction mechanism for polyprotein cleavage has been proposed by 3CL pro [43]. In the first step, the catalytic double Cys-thiol is released



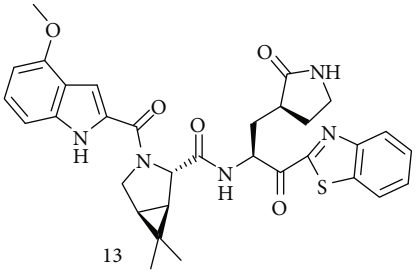
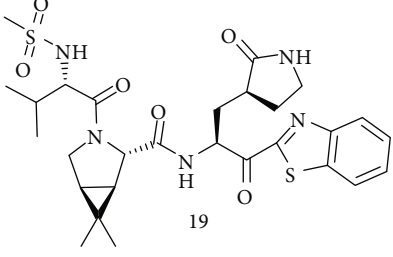
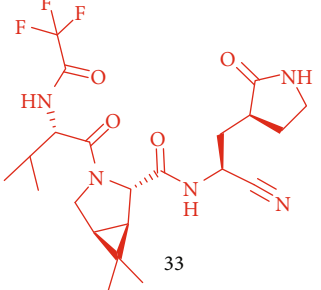
SCHEME 6: Synthesis path of compound (33).

from the proton with the help of adjacent histidine. In the next step, the anionic sulfur strikes the carbonyl carbon of the scissile amide bond. A peptide product is then dropped with an amine terminus; on the other hand, the histidine recovers the deprotonated form (step III). Finally, the thioester produced to release carboxylic acid (IV) is hydrolyzed and the catalytic binary returns to its original state, preparing it for the next proteolytic cycle (Scheme 7).

5. Safety of Nirmatrelvir in Patients Infected with the Coronavirus

Pfizer Company reports that the oral antiviral drug nirmatrelvir notably reduces the acceptance and mortality of patients with COVID-19 at higher risk for severe disease than placebo. Brief analysis of the data obtained in phases II and III showed that 1219 adults had registered by September 29, 2021. The results obtained among participants who

TABLE 1: The influence of various reaction parameters on the selectivity and efficiency of synthetic compounds.

Compounds	Temperature	Time	Solvent	pH	Yield	Ref.
 13	4°C	18 h	(100% DMSO) for a molar ratio of approximately 1 : 7	6.00	18.4 mg, 30.7 μmol, 32%	[14]
 19	4°C	18 h	(100% DMSO) for a molar ratio of approximately 1 : 5	7.00	18 mg, 30 μmol, 21%	[14]
 33	4°C	24 h	—	6.00	160.93 g, 322 mmol, 97%	[14]

were treated for 3 days after the onset of symptoms of COVID-19 showed that the risk of hospitalization for COVID-19 or death in the nirmatrelvir group was 89% lower than that in the placebo group. In the study, trial participants were randomly selected 1 : 1, half of whom received nirmatrelvir and the other half orally placebo for five days every 12 hours. Among those treated within three days of the onset of symptoms, 0.8% (3.389) of patients who received nirmatrelvir were hospitalized until the 28th day after randomization without death. In comparison, 7% (27.385) of placebo patients were hospitalized with seven deaths. A similar decrease was observed in patients treated within five days of the onset of symptoms, with 1% (6.607) in the nirmatrelvir group by day 28 (without death) and 6.7% (41.612) in the placebo group (10 deaths). In general, by day 28, no casualties were informed among patients receiving nirmatrelvir, while 10 (1.6%) in the placebo group lost their lives (Figure 5) [25, 30, 47, 48]. In another study, high confidence was obtained in the selection of the 300 mg nirmatrelvir diet in combination with the 100 mg ritonavir as BID over a 5-day term for phase 2/3 clinical trials in patients with COVID-19 [49]. Older people are at higher risk for severe complications from COVID-19. Explanation of PIMs before COVID-19 infection can increase the relation of older people that can safely be given nirmatrelvir-

ritonavir, in addition to the ordinary advantages seen with drug administration [50–52].

6. Molecular Docking of PF-07321332 (or Nirmatrelvir) and Ritonavir with 3CL^{pro}

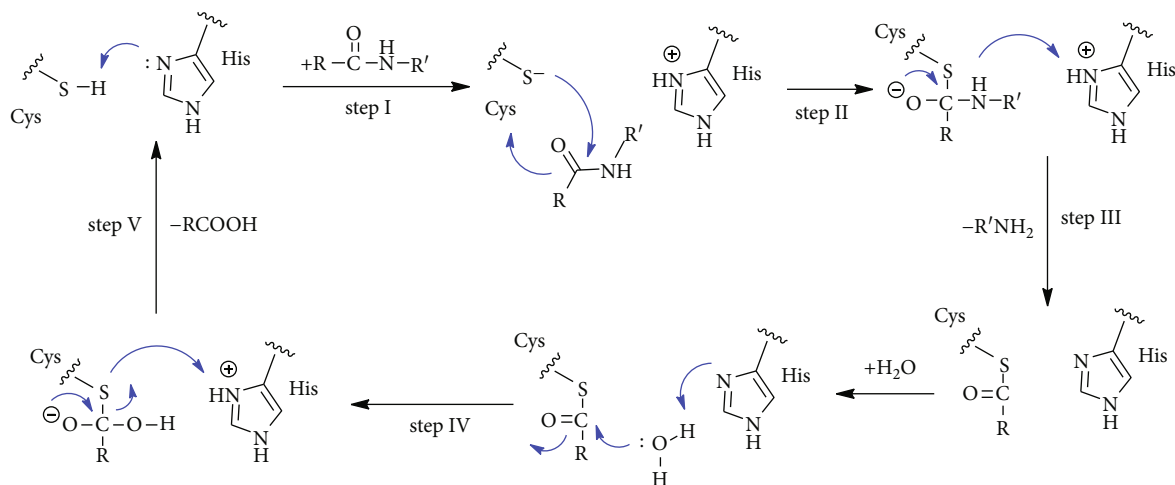
The *molecular docking* studies can be applied to model the interaction among a specific protein and small molecule at the atomic level, which helps us to determine the behavior of small molecules in the binding site of the target protein [53, 54]. In a study, the binding of PF-07321332 and ritonavir to SARS-CoV-2 3CL^{pro} was investigated by molecular dynamic (MD) simulation and molecular mechanics Poisson-Boltzmann surface area (MMPBSA) calculation [27]. α -Ketoamide and PF-07321332 (NCT04756531) molecules, which in a particular bind to and prevent SARS-CoV-2 3CL^{pro}, could be promising alternatives for fighting the epidemic [55]. A relative binding state investigation of PF-07321332 and ritonavir may supply an overview of the design of rational drugs by modifying inhibitors based on residues in the enzyme-active site. Molecular docking of PF-07321332 and ritonavir to 3CL^{pro} was performed to resolve the mechanism of binding these molecules. By the docking score and the best ligand binding mode, the best docking solutions for PF-07321332 and ritonavir were

(a)

	P4	P3	P2	P1	P1'	P2'	P2''	P4'
VP2-VP3	A	I	T	Q	G	V	P	T
VP3-VP1	D	I	A	Q	L	D	H	L
2A-2B	A	M	E	Q	G	I	T	D
2B-2C	V	P	R	Q	S	S	E	W
2C-3A	A	L	F	Q	G	P	P	Q
3A-3B	A	G	I	Q	G	P	Y	T
3B-3C	A	K	V	Q	G	P	G	F
3C-3D	T	D	T	Q	G	E	I	V

(b)

FIGURE 4: Enterovirus 3C protease (3C^{Pro}). (a) Schematic exhibition of the enterovirus polyprotein, with 3C^{Pro} division sites displayed by arrowheads. (b) Amino acid succession of polyprotein division sites. The protease splits among P1-P1'.



SCHEME 7: Schematic representation of the catalytic mechanism for the division of polyproteins by 3CL^{Pro}.

chosen. To realize the binding mechanism of PF-07321332, combined with the antiretroviral drug ritonavir to 3CL^{Pro} were done MD simulations for 100 ns. The RMSD of 3CL^{Pro} was shown to be more stable than with ritonavir associated with either PF-07321332. Unlike other systems, 3CL^{Pro} in complex with ritonavir significantly deviates between 88 and 95 ns from the simulation. PF-07321332 ligand with 3CL^{Pro} is more stable than ritonavir because the ligand was later found to deviate during the simulation. The latest clinical trials have also shown that

these antiretroviral drugs are unsuccessful because they do not significantly accelerate clinical improvement in severe COVID-19 patients [56]. RMSF 3CL^{Pro} was analyzed in all two complexes to evaluate the effect of ligand binding on protein residues. Amino acid residues 45–65 with ritonavir displayed more significant fluctuations than apo-form. These oscillation zones are mainly composed of junctional residues that are connected by a hydrogen bonding network and are involved in the formation of the catalytic pair between Cys145 and His41 [57–59].

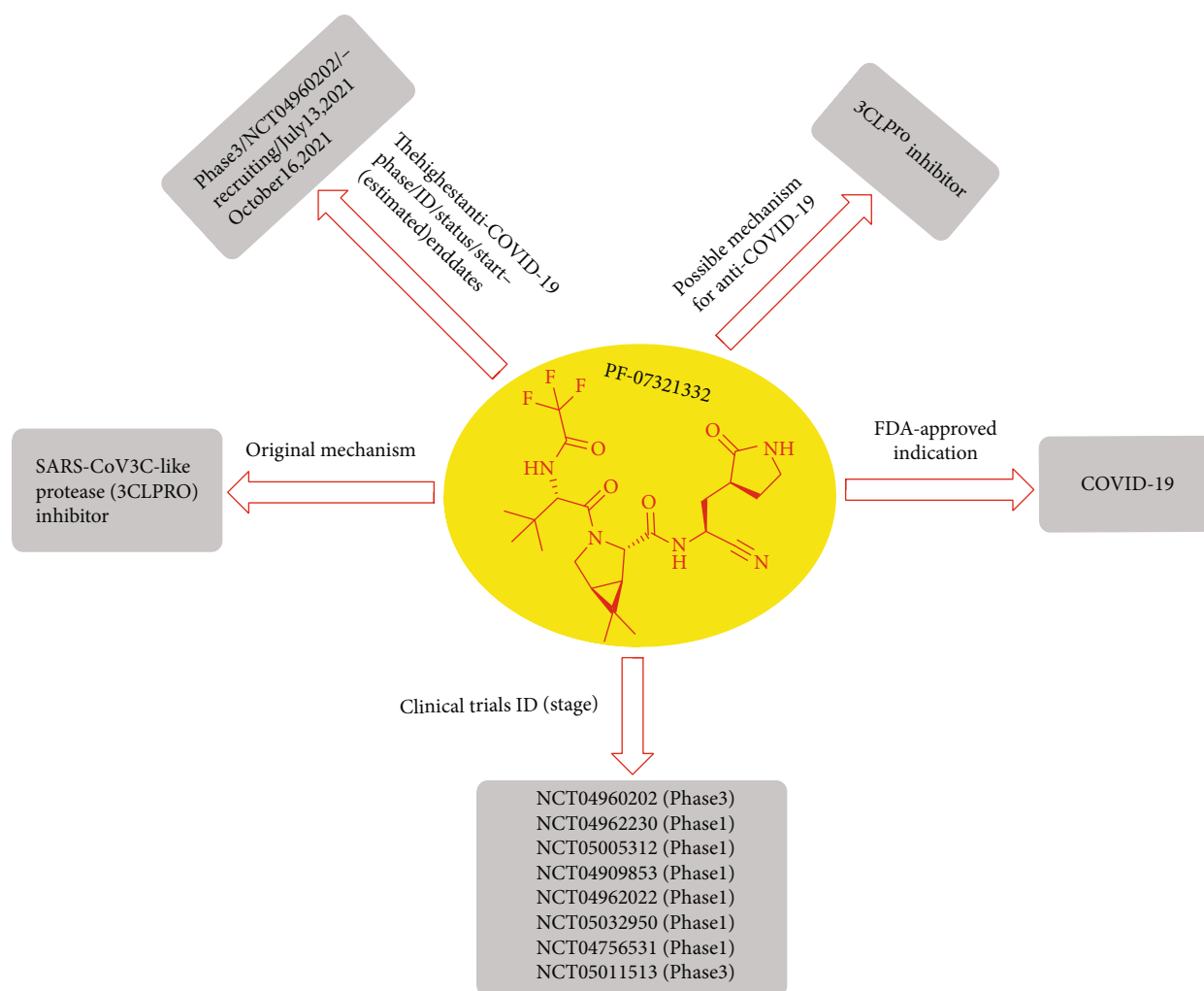


FIGURE 5: Diagram related to general specification nirmatrelvir.

3CL^{pro}-PF in the RMSF of the protein revealed the same tendency as its apo-form. PF-07321332 displayed more powerful interactions with 3CL^{pro} (in the interaction analysis), and these interactions during the simulation stayed intact because between the interacting groups, the minimum distance remained without change. The interactions (Cys145-H9 and Cys145-O2) in 3CL^{pro}-PF during the simulation stayed intact. This indicated that PF-07321332 is firmly attached to 3CL^{pro} [60]. Gly143 SARS-CoV-2 3CL^{pro} has been reported as the most desirable residue for forming hydrogen bonds with ligands, followed by Cys145, His163, and Glu166 (Table 2) [61]. In similar research, the recognized compounds bind to the similar binding site but the resistance of the complex is less than the one of PF-07321332 [62, 63]. Given the current epidemic position, it is essential to find a potent candidate drug with desirable binding affinity.

Macchiagodena et al. [64] have investigated the noncovalent interaction between PF-07321332 and SARS-CoV-2 main protease at the atomic level using a computational approach based on extensive molecular dynamics simulations with explicit solvent. This drug, in mixture with ritona-

TABLE 2: Residue and ligand atom interactions with the bond type and energy in 3CL^{pro} complexes.

Complex	Residue	Ligand atom	Bond type	Energy
3CL ^{pro} -PF	Cys145	O ₂	Hydrogen	-5.04
	Cys145	H ₉	Hydrogen	-5.04
	Glu166	O ₃	Hydrogen	-4.41
	Gln189	H ₂ O	Hydrogen	-3.01
3CL ^{pro} -rit	His41	H ₆	Arene	-3.0
	Gly143	O ₃	Hydrogen	-2.2
	Pro168	Thiozyl group	Arene	-0.8
	Thr190	S ₂	Hydrogen	-0.8

vir, depends on the electrophilic invasion of a nitrile cap to the catalytic cysteine of the protease. Nonbonding interactions between the residues of the binding pocket and the inhibitor and with water molecules on the protein level have been determined by the two possible protonation states and two distinct force fields of the major protease catalytic binary HIS41-CYS145. When the catalytic binary is in the impartial

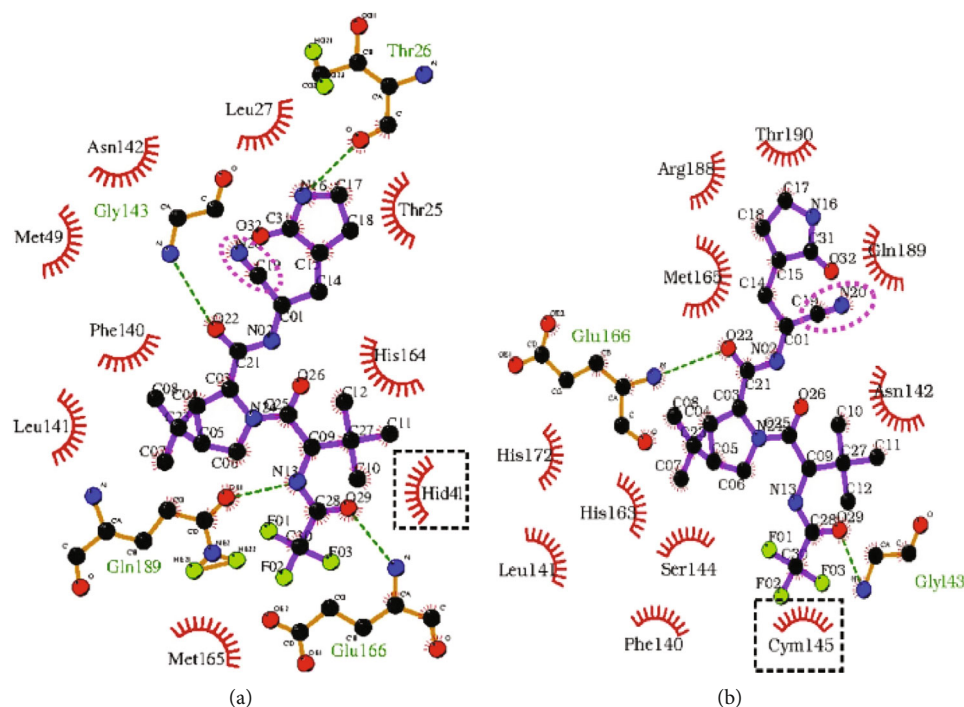


FIGURE 6: Two-dimensional display of the best docking poses at the connection achieved with LIGPLOT [68]. The nitrile group is a circular magenta color. The hydrogen bonds with dashed green lines and the binary residues are shown with black dashed squares. (a) Dyad in the neutral condition. (b) Dyad in zwitterionic condition.

state, the noncovalent bond is probably stronger. Simulation (MD) appears to hold up a two-step inhibitory mechanism: (a) adding noncovalently paired to a neutral form and (b) thiolate-imidazolium formation and ligand displacement to finalize the electrophilic attack (Figure 6) [64].

PF-07321332 reacts with a cysteine residue at the protease binding site to stop viral repetition in cells (as a 3CL^{PRO} inhibitor). This cysteine plays an important part in the activity of 3CL^{PRO} coronaviruses. Thus, inhibition of activity prevents the encoding of the viral genome of functional NSPs and ultimately suppresses virus replication [44, 65]. PF-07321332, with its nitrile group, acts as a polypeptide covalent inhibitor. Peptide nitriles react with catalytic cysteine as site-specific reversible inhibitors to form sulfides. This inhibitor provides a γ -lactam ring, a usual attribute of most covalent inhibitors developed against SARS-CoV-2 3CL^{PRO}, which utilizes the selectivity of glutamine residues in the cleft multiprotein equivalent site [66, 67].

7. Comparison of the Performance of Two New Oral Antiviruses (Molnupiravir and Nirmatrelvir) for the Treatment of COVID-19

Although the coronavirus disease epidemic has not been fully controlled but significant advances have been made in COVID-19 studies [1, 2] and antiviral drugs have displayed good therapeutic results against COVID-19, an easy oral antiviral drug for COVID-19 has not yet been expanded. Molnupiravir is a small molecular prodrug of the nucleoside

derivative *N*-hydroxycytidine (NHC). NHC triphosphate attacks viral RNA polymerase and has been demonstrated to prevent the repetition of several RNA viruses containing SARS-CoV-2 [69, 70]. Considerable clinical advantages have been shown in a vast phase 3 clinical trial for COVID-19 patients distributed orally with molnupiravir [71]. This is the first accepted oral antiviral factor for the treatment of COVID-19. MTP can be employed by the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 instead of cytidine triphosphate (CTP) or uracil triphosphate (UTP) as a substrate. First, RdRp probably develops the combination of molnupiravir instead of C or U in the synthesis of positive-strand genomic RNA that acts as a template for the synthesis of negative-strand genomic RNA and subgenomic RNA. In the next step, the negative-strand genomic RNA comprising molnupiravir can be applied as a sample for the synthesis of positive-strand genomic RNA or positive-stranded subgenomic mRNA [72]. Paxlovid is composed of the protease inhibitor PF-07321332 (designed specifically for the protease SARS-CoV-2-3CL) and ritonavir (used in HIV therapy). The purpose of ritonavir is to increase the effectiveness of an oral protease inhibitor by stopping the rapid metabolism of PF-07321332 using liver enzymes, so maintaining a sufficient circulating concentration of PF-07321332 to inactivate the virus [73]. PF-07321332 is responsible for the cleavage of precursor proteins to structural proteins and enzymes that act on virus replication and maturation by inhibiting viral proteolysis mediated by SARS-CoV-2-3CL protease [73–76].

In the other study, nirmatrelvir (PF-07321332), and other clinically relevant SARS-CoV-2 antivirals, were tested against a panel of SARS-CoV-2 variants, including the novel

Omicron variant, in live-virus antiviral assays. [77]. Rosales [77] confirmed that nirmatrelvir and other clinically related antiviral drugs all retain their activity against all types tested, including Omicron.

Li et al. [78] have shown that molnupiravir and nirmatrelvir strongly inhibit SARS-CoV-2 Omicron infection. A combination of molnupiravir and nirmatrelvir applied synergistic antiviral property. Remarkably, there are delicate differences in antiviral response patterns between the Omicron, WT, and Delta variants, also between cell lines and organoid patterns. However, these discoveries support the use of molnupiravir and nirmatrelvir in the treatment of patients with Omicron.

Omicron-type acute respiratory distress syndrome 2 (SARS-CoV-2) is currently the most prevalent type in the United States, and its numerous mutations have invalidated some previously authorized treatments [79]. The US National Institutes of Health (NIH) has published a list of drugs that should now be investigated for use (in the treatment of outpatients). Nirmatrelvir with ritonavir (Paxlovid) as one of these drugs is understood to be clinically effective and prioritized for ease of administration. For the treatment of mild to moderate COVID-19, it is essential to understand the key principles about recommended treatments. Nirmatrelvir, the antiviral active ingredient in Paxlovid, inhibits the main SARS-CoV-2 protease, Mpro, thus preventing the virus from replicating [80, 81]. Ritonavir inhibits the metabolism of nirmatrelvir by CYP3A, thereby increasing the plasma concentration of nirmatrelvir and by itself having no activity against SARS-CoV-2. Ritonavir-induced drug interactions may occur, and renal and hepatic impairment may limit the use of Paxlovid in some patients. Ritonavir is known for its use as an HIV-1 protease inhibitor, and the potential for antiretroviral resistance must be confirmed by the prescriber. Amid the limited supply of new treatments for mild to moderate COVID-19 outpatient treatment, physicians face new challenges, especially among those practicing in military medical centers abroad [82]. Although prescribers may not be familiar with these drugs, their proper use requires careful examination of the patient's symptoms and familiarity with the characteristics of the side effects of each drug. Given these complexities, a step-by-step guide is provided here to assist physicians in managing outpatients with mild to moderate COVID-19 by nirmatrelvir with ritonavir (Paxlovid) as one of these effective drugs.

8. Conclusions

Nowadays, the COVID-19 pandemic is one of the biggest challenges which is the leading cause of death in many countries in the globe. Therefore, the discovery and development of new drugs for novel coronavirus (CoV) are essential. This review is focused on summarizing recent research on nirmatrelvir. It will aid researchers, organic chemists, medicinal chemists, pharmacologists, etc., in the discovery and the synthesis of the new effective antiviral compounds. Nirmatrelvir as an orally antiviral agent displayed a promising combination for nonhospitalized patients with COVID-19.

Abbreviations

BID:	Nirmatrelvir/ritonavir twice daily
3CL ^{pro} :	3-Chymotrypsin-like protease
CH ₃ CN:	Acetonitrile
CH ₃ OH:	Methanol
CTP:	Cytidine triphosphate
Cys145:	Cysteine
DCM, CH ₂ Cl ₂ :	Dichloromethane
DIEA:	<i>N,N</i> -Diisopropylethylamine, Hunig's base
DMF:	<i>N,N</i> -Dimethylformamide
EDC, EDAC or	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDCI:	Triethylamine
Et ₃ N:	Diethyl ether
Et ₂ O:	Ethyl acetate
EtOAc:	O-(7-Azabenzotriazol-1-yl)- <i>N,N,N,N</i> -tetramethyluronium hexafluorophosphate
HATU:	Hydrogen chloride
HCl:	Hexafluoroisopropanol
HFIP:	Histidine
His:	2-Hydroxypyridine 1-oxide
HOPO:	Isopropanol
IPA:	Isopropyl acetate
IPAC:	Lithium hydroxide
LiOH:	Molecular dynamic
MD:	Methyl ethyl ketone, butan-2-one
MEK:	Molecular mechanics
MMPBSA:	Poisson-Boltzmann surface area
M ^{pro} :	Main protease
MTBE:	Methyl tert-butyl ether
NaCl:	Sodium chloride
NHC:	<i>N</i> -Hydroxycytidine
NSPs:	Nonstructural proteins
PI:	Protease inhibitor
PIMs:	Potentially inappropriate medications
PL ^{pro} :	Papain-like protease
RdRp:	RNA-dependent RNA polymerase
RMSD:	Root mean square deviation
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2
TFA:	Trifluoroacetic acid
TsOH:	Para-toluenesulfonate
UTP:	Uracil triphosphate
WT:	Wild type.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

The ethical committee has ethically approved this research: IR.FUMS.REC.1400.105.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

The first draft of the manuscript was written and revised and edited by M. M. Also, M. V. and M. B. revised and edited the first draft of the manuscript. The conception and design of the study were done by E. Z. Also, the first draft of the manuscript was written and revised and edited by E. Z.

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References

- [1] E. Zarenezhad and M. Marzi, "Review on molnupiravir as a promising oral drug for the treatment of COVID-19," *Medicinal Chemistry Research*, vol. 31, no. 2, pp. 232–243, 2022.
- [2] E. Zarenezhad, S. Behrouz, M. Farjam, and M. N. S. Rad, "A mini review on discovery and synthesis of remdesivir as an effective and promising drug against COVID-19," *Russian Journal of Bioorganic Chemistry*, vol. 47, no. 3, pp. 609–621, 2021.
- [3] O. Cruz-Milan and S. Lagunas-Puls, "Effects of COVID-19 on variations of taxpayers in tourism-reliant regions: the case of the Mexican Caribbean," *Journal of Risk and Financial Management*, vol. 14, no. 12, p. 578, 2021.
- [4] D. R. Owen, C. M. N. Allerton, A. S. Anderson et al., "An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19," *Science*, vol. 374, no. 6575, pp. 1586–1593, 2021.
- [5] K. Anand, J. Ziebuhr, P. Wadhvani, J. R. Mesters, and R. Hilgenfeld, "Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs," *Science*, vol. 300, no. 5626, pp. 1763–1767, 2003.
- [6] P. S. Dragovich, T. J. Prins, R. Zhou et al., "Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 4. Incorporation of P1 lactam moieties as L-glutamine replacements," *Journal of Medicinal Chemistry*, vol. 42, no. 7, pp. 1213–1224, 1999.
- [7] A. Harreus, R. Backes, J. O. Eichloer et al., *2-Pyrrolidone*, Wiley-VCH Verlag GmbH & Co, 2011.
- [8] M. Pavan, G. Bolcato, D. Bassani, M. Sturlese, and S. Moro, "Supervised molecular dynamics (SuMD) insights into the mechanism of action of SARS-CoV-2 main protease inhibitor PF-07321332," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 36, no. 1, pp. 1646–1650, 2021.
- [9] E. Mahase, *Myanmar: Medical organisations unite to condemn attacks on healthcare workers*, British Medical Journal Publishing Group, 2021.
- [10] B. Boras, R. M. Jones, B. J. Anson et al., "Discovery of a novel inhibitor of coronavirus 3CL protease as a clinical candidate for the potential treatment of COVID-19," *BioRxiv*, pp. 2009–2020, 2021.
- [11] R. L. Hoffman, R. S. Kania, M. A. Brothers et al., "Discovery of ketone-based covalent inhibitors of coronavirus 3CL proteases for the potential therapeutic treatment of COVID-19," *Journal of Medicinal Chemistry*, vol. 63, no. 21, pp. 12725–12747, 2020.
- [12] Y.-P. Hung, J. C. Lee, C. W. Chiu et al., "Oral nirmatrelvir/ritonavir therapy for COVID-19: the dawn in the dark?," *Antibiotics*, vol. 11, no. 2, p. 220, 2022.
- [13] L. Zhang, D. Lin, Y. Kusov et al., "α-Ketoamides as broad-spectrum inhibitors of coronavirus and enterovirus replication: structure-based design, synthesis, and activity assessment," *Journal of medicinal chemistry*, vol. 63, no. 9, pp. 4562–4578, 2020.
- [14] D. R. Owen, C. M. N. Allerton, A. S. Anderson et al., "An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19," *Science*, vol. 374, no. 6575, pp. 1586–1593, 2021.
- [15] N. Catlin, C. J. Bowman, S. N. Campion et al., "Reproductive and developmental safety of nirmatrelvir (PF-07321332), an oral SARS-CoV-2 M^{pro} inhibitor in animal models," *Reproductive Toxicology*, vol. 108, pp. 56–61, 2022.
- [16] J. Hammond, H. Leister-Tebbe, A. Gardner et al., "Oral nirmatrelvir for high-risk, nonhospitalized adults with COVID-19," *New England Journal of Medicine*, vol. 386, no. 15, pp. 1397–1408, 2022.
- [17] J. Couzin-Frankel, "Antiviral pills could change pandemic's course," *American Association for the Advancement of Science*, vol. 374, no. 6569, pp. 799–800.
- [18] K. Fernando, S. Menon, K. Jansen et al., "Achieving end-to-end success in the clinic: Pfizer's learnings on R&D productivity," *Drug Discovery Today*, vol. 27, no. 3, 2022.
- [19] E. G. McDonald and T. C. Lee, "Nirmatrelvir-ritonavir for COVID-19," *CMAJ*, vol. 194, no. 6, pp. E218–E218, 2022.
- [20] S. Ullrich, K. B. Ekanayake, G. Otting, and C. Nitsche, "Main protease mutants of SARS-CoV-2 variants remain susceptible to nirmatrelvir," *Bioorganic & Medicinal Chemistry Letters*, vol. 62, article 128629, 2022.
- [21] B. Halford, "Pfizer unveils its oral SARS-CoV-2 inhibitor," *Chemical & Engineering News*, vol. 99, no. 13, pp. 7–7, 2021.
- [22] N. J. Vickers, "Animal communication: when I'm calling you, will you answer too?," *Current Biology*, vol. 27, no. 14, pp. R713–R715, 2017.
- [23] M. N. S. Rad, S. Behrouz, E. Zarenezhad, and N. Kaviani, "Highly efficient protocol for one-pot N-alkylation of nucleobases using alcohols in bmim [Br]: a rapid route to access acyclic nucleosides," *Journal of the Iranian Chemical Society*, vol. 12, no. 9, pp. 1603–1612, 2015.
- [24] P. Thanigaimalai, S. Konno, T. Yamamoto et al., "Development of potent dipeptide-type SARS-CoV 3CL protease inhibitors with novel P3 scaffolds: design, synthesis, biological evaluation, and docking studies," *European Journal of Medicinal Chemistry*, vol. 68, pp. 372–384, 2013.
- [25] K. Vandyck and J. Deval, "Considerations for the discovery and development of 3-chymotrypsin-like cysteine protease inhibitors targeting SARS-CoV-2 infection," *Current Opinion in Virology*, vol. 49, pp. 36–40, 2021.
- [26] S. Yavuz and S. Unal, "Antiviral treatment of COVID-19: an update," *Turkish Journal of Medical Sciences*, vol. 50, no. 9, pp. 611–619, 2021.
- [27] B. Ahmad, M. Batool, Q. . Ain, M. S. Kim, and S. Choi, "Exploring the binding mechanism of PF-07321332 SARS-CoV-2 protease inhibitor through molecular dynamics and binding free energy simulations," *International Journal of Molecular Sciences*, vol. 22, no. 17, p. 9124, 2021.

- [28] Covid-19, P b d i p I t o a d f, "Clinical Trials Arena," 2021.
- [29] L. Müller, M. André, W. Moskorz et al., "Age-dependent immune response to the biontech/pfizer BNT162b2 coronavirus disease 2019 vaccination," *Clinical Infectious Diseases*, vol. 73, no. 11, pp. 2065–2072, 2021.
- [30] E. Mahase, "COVID-19: Pfizer's paxlovid is 89% effective in patients at risk of serious illness, company reports," *British Medical Journal Publishing Group*, vol. 375, p. n2713.
- [31] M. W., *What is Australia's potential new COVID treatment?*, The Royal Australian College of General Practitioners (RACGP), 2021.
- [32] E. Dong, H. Du, and L. Gardner, "An interactive web-based dashboard to track COVID-19 in real time," *The Lancet Infectious Diseases*, vol. 20, no. 5, pp. 533–534, 2020.
- [33] Y. Yang, F. Peng, R. Wang et al., "The deadly coronaviruses: the 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China," *Journal of Autoimmunity*, vol. 109, article 102434, 2020.
- [34] V. G. da Costa, M. L. Moreli, and M. V. Saivish, "The emergence of SARS, MERS and novel SARS-2 coronaviruses in the 21st century," *Archives of Virology*, vol. 165, no. 7, pp. 1517–1526, 2020.
- [35] C. Wang, P. W. Horby, F. G. Hayden, and G. F. Gao, "A novel coronavirus outbreak of global health concern," *The Lancet*, vol. 395, no. 10223, pp. 470–473, 2020.
- [36] J. Ziebuhr, "Molecular biology of severe acute respiratory syndrome coronavirus," *Current Opinion in Microbiology*, vol. 7, no. 4, pp. 412–419, 2004.
- [37] S. Ullrich and C. Nitsche, "The SARS-CoV-2 main protease as drug target," *Bioorganic & Medicinal Chemistry Letters*, vol. 30, no. 17, article 127377, 2020.
- [38] V. Grum-Tokars, K. Ratia, A. Begaye, S. C. Baker, and A. D. Mesecar, "Evaluating the 3C-like protease activity of SARS-coronavirus: recommendations for standardized assays for drug discovery," *Virus Research*, vol. 133, no. 1, pp. 63–73, 2008.
- [39] N. Zhong, S. Zhang, P. Zou et al., "Without its N-finger, the main protease of severe acute respiratory syndrome coronavirus can form a novel dimer through its C-terminal domain," *Journal of Virology*, vol. 82, no. 9, pp. 4227–4234, 2008.
- [40] B. Xia and X. Kang, "Activation and maturation of SARS-CoV main protease," *Protein & Cell*, vol. 2, no. 4, pp. 282–290, 2011.
- [41] B.-L. Ho, S. C. Cheng, L. Shi, T. Y. Wang, K. I. Ho, and C. Y. Chou, "Critical assessment of the important residues involved in the dimerization and catalysis of MERS coronavirus main protease," *PLoS One*, vol. 10, no. 12, article e0144865, 2015.
- [42] T. Pillaiyar, M. Manickam, V. Namasivayam, Y. Hayashi, and S. H. Jung, "An overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease inhibitors: peptidomimetics and small molecule chemotherapy," *Journal of Medicinal Chemistry*, vol. 59, no. 14, pp. 6595–6628, 2016.
- [43] C. Huang, P. Wei, K. Fan, Y. Liu, and L. Lai, "3C-like proteinase from SARS coronavirus catalyzes substrate hydrolysis by a general base mechanism," *Biochemistry*, vol. 43, no. 15, pp. 4568–4574, 2004.
- [44] T. Muramatsu, C. Takemoto, Y. T. Kim et al., "SARS-CoV 3CL protease cleaves its C-terminal autoprocessing site by novel subsite cooperativity," *Proceedings of the National Academy of Sciences*, vol. 113, no. 46, pp. 12997–13002, 2016.
- [45] S. A. Amin, S. Banerjee, K. Ghosh, S. Gayen, and T. Jha, "Protease targeted COVID-19 drug discovery and its challenges: insight into viral main protease (Mpro) and papain-like protease (PLpro) inhibitors," *Bioorganic & Medicinal Chemistry*, vol. 29, article 115860, 2021.
- [46] R. Liang, L. Wang, N. Zhang et al., "Development of small-molecule MERS-CoV inhibitors," *Viruses*, vol. 10, no. 12, p. 721, 2018.
- [47] S. Hu, S. Jiang, X. Qi, R. Bai, X. Y. Ye, and T. Xie, "Races of small molecule clinical trials for the treatment of COVID-19: an up-to-date comprehensive review," *Drug Development Research*, vol. 83, no. 1, pp. 16–54, 2022.
- [48] E. Mahase, "COVID-19: UK stockpiles two unapproved antiviral drugs for treatment at home," *British Medical Journal Publishing Group*, vol. 375, p. n2602, 2021.
- [49] R. S. P. Singh, S. S. Toussi, F. Hackman et al., "Innovative randomized phase I study and dosing regimen selection to accelerate and inform pivotal COVID-19 trial of nirmatrelvir," *Clinical Pharmacology & Therapeutics*, vol. 112, no. 1, pp. 101–111, 2022.
- [50] S. Ross, E. Bortolussi-Courval, R. Hanula, T. C. Lee, M. G. Wilson, and E. G. McDonald, "COVID-SAFER: deprescribing guidance for nirmatrelvir-ritonavir drug interactions in older adults," *medRxiv*, 2022.
- [51] D. M. Salerno, D. L. Jennings, N. W. Lange et al., "Early clinical experience with nirmatrelvir/ritonavir for the treatment of COVID-19 in solid organ transplant recipients," *American Journal of Transplantation*, 2022.
- [52] H. Eng, A. L. Dantonio, E. P. Kadar et al., "Disposition of nirmatrelvir, an orally bioavailable inhibitor of SARS-CoV-2 3C-like protease, across animals and humans," *Drug Metabolism and Disposition*, vol. 50, no. 5, pp. 576–590, 2022.
- [53] M. H. Afsarian, M. Farjam, E. Zarenezhad, S. Behrouz, and M. N. S. Rad, "Synthesis, antifungal evaluation and molecular docking studies of some tetrazole derivatives," *Acta Chimica Slovenica*, vol. 66, no. 4, pp. 874–887, 2019.
- [54] B. J. McConkey, V. Sobolev, and M. Edelman, "The performance of current methods in ligand-protein docking," *Current Science*, pp. 845–856, 2002.
- [55] L. Zhang, D. Lin, X. Sun et al., "Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α -ketoamide inhibitors," *Science*, vol. 368, no. 6489, pp. 409–412, 2020.
- [56] B. Cao, Y. Wang, D. Wen et al., "A trial of lopinavir-ritonavir in adults hospitalized with severe COVID-19," *New England Journal of Medicine*, vol. 382, no. 19, pp. 1787–1799, 2020.
- [57] M. T. Ul Qamar, S. M. Alqahtani, M. A. Alamri, and L. L. Chen, "Structural basis of SARS-CoV-2 3CL^{Pro} and anti-COVID-19 drug discovery from medicinal plants," *Journal of Pharmaceutical Analysis*, vol. 10, no. 4, pp. 313–319, 2020.
- [58] A. Paasche, A. Zipper, S. Schäfer, J. Ziebuhr, T. Schirmeister, and B. Engels, "Evidence for substrate binding-induced zwitterion formation in the catalytic Cys-His dyad of the SARS-CoV main protease," *Biochemistry*, vol. 53, no. 37, pp. 5930–5946, 2014.
- [59] X. Xue, H. Yu, H. Yang et al., "Structures of two coronavirus main proteases: implications for substrate binding and antiviral drug design," *Journal of Virology*, vol. 82, no. 5, pp. 2515–2527, 2008.
- [60] H. M. Mengist, T. Dilnessa, and T. Jin, "Structural basis of potential inhibitors targeting SARS-CoV-2 main protease," *Chemistry*, vol. 9, 2021.

- [61] D. D. Nguyen, K. Gao, J. Chen, R. Wang, and G. W. Wei, "Unveiling the molecular mechanism of SARS-CoV-2 main protease inhibition from 137 crystal structures using algebraic topology and deep learning," *Chemical Science*, vol. 11, no. 44, pp. 12036–12046, 2020.
- [62] A. Uniyal, M. K. Mahapatra, V. Tiwari, R. Sandhir, and R. Kumar, "Targeting SARS-CoV-2 main protease: structure based virtual screening, in silico ADMET studies and molecular dynamics simulation for identification of potential inhibitors," *Journal of Biomolecular Structure and Dynamics*, vol. 40, no. 8, pp. 3609–3625, 2022.
- [63] T. S. Ram, M. Munikumar, V. N. Raju et al., "In silico evaluation of the compounds of the ayurvedic drug, AYUSH-64, for the action against the SARS-CoV-2 main protease," *Journal of Ayurveda and Integrative Medicine*, vol. 13, no. 1, p. 100413, 2022.
- [64] M. Macchiagodena, M. Pagliai, and P. Procacci, "Characterization of the non-covalent interaction between the PF-07321332 inhibitor and the SARS-CoV-2 main protease," *Journal of Molecular Graphics and Modelling*, vol. 110, article 108042, 2022.
- [65] M. I. Ionescu, "An overview of the crystallized structures of the SARS-CoV-2," *The Protein Journal*, vol. 39, no. 6, pp. 600–618, 2020.
- [66] C. A. Ramos-Guzmán, J. J. Ruiz-Pernía, and I. Tuñón, "Computational simulations on the binding and reactivity of a nitrile inhibitor of the SARS-CoV-2 main protease," *Chemical Communications*, vol. 57, no. 72, pp. 9096–9099, 2021.
- [67] L. Zhao, S. Li, and W. Zhong, "Mechanism of action of small-molecule agents in ongoing clinical trials for SARS-CoV-2: a review," *Frontiers in Pharmacology*, vol. 13, pp. 840639–840639, 2022.
- [68] A. C. Wallace, R. A. Laskowski, and J. M. Thornton, "LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions," *Protein Engineering, Design and Selection*, vol. 8, no. 2, pp. 127–134, 1995.
- [69] Y. Wang, P. Li, K. Solanki et al., "Viral polymerase binding and broad-spectrum antiviral activity of molnupiravir against human seasonal coronaviruses," *Virology*, vol. 564, pp. 33–38, 2021.
- [70] A. Wahl, L. E. Gralinski, C. E. Johnson et al., "SARS-CoV-2 infection is effectively treated and prevented by EIDD-2801," *Nature*, vol. 591, no. 7850, pp. 451–457, 2021.
- [71] A. Jayk Bernal, M. M. Gomes da Silva, D. B. Musungaie et al., "Molnupiravir for oral treatment of COVID-19 in nonhospitalized patients," *New England Journal of Medicine*, vol. 386, no. 6, pp. 509–520, 2022.
- [72] F. Kabinger, C. Stiller, J. Schmitzová et al., "Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis," *Nature Structural & Molecular Biology*, vol. 28, no. 9, pp. 740–746, 2021.
- [73] L. B. Teixeira and W. C. Santos, "Molnupiravir and paxlovid—how much is genomic and protein destabilization harmful to SARS-CoV-2 survival and resistance?," *Actualidad en farmacología y terapéutica*, vol. 19, no. 4, pp. 235–239, 2021.
- [74] D. A. Sabbah, R. Hajjo, S. K. Bardaweel, and H. A. Zhong, "An updated review on SARS-CoV-2 main Proteinase (MPro): protein structure and small-molecule inhibitors," *Current Topics in Medicinal Chemistry*, vol. 21, no. 6, pp. 442–460, 2021.
- [75] W. Wen, C. Chen, J. Tang et al., "Efficacy and safety of three new oral antiviral treatment (molnupiravir, fluvoxamine and Paxlovid) for COVID-19: a meta-analysis," *Annals of Medicine*, vol. 54, no. 1, pp. 516–523, 2022.
- [76] J. Reina and C. Iglesias, "Nirmatrelvir más ritonavir (Paxlovid) una potente combinación inhibidora de la proteasa 3CLpro del SARS-CoV-2," *Revista Española de Quimioterapia*, vol. 35, no. 3, p. 236.
- [77] R. Rosales, B. L. McGovern, M. L. Rodriguez et al., "Nirmatrelvir, Molnupiravir, and Remdesivir maintain potent in vitro activity against the SARS-CoV-2 Omicron variant," *bioRxiv*, 2022.
- [78] P. Li, Y. Wang, M. Lavrijsen et al., "SARS-CoV-2 Omicron variant is highly sensitive to molnupiravir, nirmatrelvir, and the combination," *Cell Research*, vol. 32, no. 3, pp. 322–324, 2022.
- [79] S. A. Madhi, G. Kwatra, J. E. Myers et al., "Population immunity and Covid-19 severity with Omicron variant in South Africa," *Vaccination Demographics Trends*, vol. 386, no. 14, pp. 1314–1326, 2022.
- [80] Food, U and D Administration, "Fact sheet for healthcare providers: emergency use authorization for Paxlovid," *Recuperada de*, vol. 17, no. 1, 2021 <https://www.fda.gov/media/155050/download>.el.
- [81] C. Fenton and S. J. Keam, "Emerging small molecule antivirals may fit neatly into COVID-19 treatment," *Drugs & Therapy Perspectives*, vol. 38, no. 3, pp. 112–126, 2022.
- [82] D. S. Krauth, "Clinical pathways when considering antimicrobials for the treatment of mild-to-moderate COVID-19," *Military Medicine*, 2022.