Medicinal Chemistry Research (2022) 31:1637–1646
https://doi.org/10.1007/s00044-022-02951-6

MEDICINAL CHEMISTRY RESEARCH

REVIEW ARTICLE

The history, mechanism, and perspectives of nirmatrelvir (PF-07321332): an orally bioavailable main protease inhibitor used in combination with ritonavir to reduce COVID-19-related hospitalizations

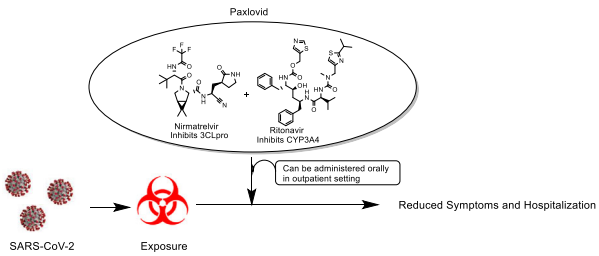
Ryan P. Joyce[1] · Vivian W. Hu[1] · Jun Wang[1]

Received: 23 June 2022 / Accepted: 15 August 2022 / Published online: 30 August 2022

Abstract

The rapid development of effective vaccines to combat the SARS-CoV-2 virus has been an effective counter measure to decrease hospitalization and the mortality rate in many countries. However, with the risk of mutated strains decreasing the efficacy of the vaccine, there has been an increasing demand for antivirals to treat COVID-19. While antivirals, such as remdesivir, have had some success treating COVID-19 patients in hospital settings, there is a need for orally bioavailable, cost-effective antivirals that can be administered in outpatient settings to minimize COVID-19-related hospitalizations and death. Nirmatrelvir (PF-07321332) is an orally bioavailable M^{pro} (also called $3CL^{pro}$) inhibitor developed by Pfizer. It is administered in combination with ritonavir, a potent CYP3A4 inhibitor that decreases the metabolism of nirmatrelvir. This review seeks to outline the history of the rational design, the target selectivity, synthesis, drug resistance, and future perspectives of nirmatrelvir.

Graphical abstract



Keywords SARS-CoV-2 · Main protease · Paxlovid · Antiviral · Nirmatrelvir

Introduction

The novel coronavirus that results in the coronavirus disease 2019 (COVID-19), identified as SARS-CoV-2, was discovered in China in December 2019; it quickly spread worldwide, infecting over 537 million people and resulting in over 6.3 million deaths as of June 20th, 2022 [1, 2]. With the emergence of variants of concern and variants of interest, there has been an increasing demand for more adaptive diagnostic methods, vaccines, and antiviral drugs [3, 4]. Unlike SARS-CoV, a large of percentage of SARS-CoV-2 infected individuals are asymptomatic,

✉ Jun Wang
junwang@pharmacy.rutgers.edu

[1] Department of Medicinal Chemistry, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

rendering it challenging for contact tracing. Even in countries where vaccination rates are high, infection rates continue to rise [4]. Therefore, it is urgent to develop orally bioavailable antiviral drugs to better combat the spread of COVID-19 [4]. Antivirals are not substituents but essential complements to vaccines.

Previously FDA approved therapeutics to treat COVID-19 include remdesivir (**1**), an RNA-dependent RNA polymerase (RdRp) inhibitor administered intravenously to COVID-19 infected adults and children over age 12 in the hospital setting [5]. Remdesivir (**1**) is unstable in blood and has a limited half-life of less than an hour in humans, therefore requiring continuous infusion [5, 6]. Other available intravenous drugs include the neutralizing monoclonal antibodies, imdevimab, bamlanivimab, etesevimab, and casirivimab [7]. However, similar to remdesivir (**1**), these drugs are costly and must be administered in a hospital setting [7]. Molnupiravir (**2**) has emerged as an orally bioavailable SARS-CoV-2 RdRp inhibitor that received full regulatory approval in the U.K. in November of 2021, further establishing the existing desire for new orally bioavailable COVID-19 drugs [7]. Many pharmaceutical companies have been developing antivirals for SARS-CoV-2, which led to the approval of nirmatrelvir (PF-07321332, **3**) [7, 8]. Nirmatrelvir (**3**) is a second-generation orally bioavailable peptidomimetic M^{pro} inhibitor developed by Pfizer. The Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients (EPIC-HR) trial and an interim study with a total of 2246 patients showed promising results with an 89% reduction in hospitalization due to COVID-19 and no deaths, compared to the placebo groups 7 deaths [7, 9, 10]. On December 22nd 2021, the FDA approved the emergency use authorization of nirmatrelvir/ritonavir combination (Paxlovid) [7, 11]. Nirmatrelvir (**3**) is a reversible covalent inhibitor and ritonavir (**4**) is included to maintain the effective concentration of nirmatrelvir (**3**) above the therapeutic level [12].

Target validation

The SARS-CoV-2 genome encodes two polyproteins, pp1a and pp1ab, and four structural proteins [13]. The polyproteins are cleaved by M^{pro} at 11 sites to yield nonstructural proteins that are vital to viral replication; preventing this cleavage would inhibit viral replication and proliferation [14–16]. M^{pro} is a cysteine protease with substrate preference for glutamine at the P1 site [17, 18]. M^{pro} is highly conserved across the *coronaviridae* family; SARS-CoV-1 M^{pro} shares ~96% amino acid sequence similarity to SARS-CoV-2 M^{pro} with an active site that is highly similar [16, 18–20]. M^{pro} is comprised of two coupled units that fold independently of each other: they are the catalytic site containing chymotrypsin-like domains I + II that contains the C145-H41 catalytic dyad (residues 1–197) and the cluster of helices domain III (residues 198–304) [19]. M^{pro} forms a dimer due to the interactions between the N-terminus of domain I + II and the C-terminus of domain III; this dimer is reversible and more stable when substrate is bound [19, 21]. No known human cysteine protease cleaves after glutamine, potentially increasing selectivity and making M^{pro} an attractive drug target [16, 22].

Due to the highly conserved M^{pro} sequence between SARS-CoV-1 and SARS-CoV-2, some previously discovered compounds developed over 15 years ago to treat SARS-CoV-1, such as PF-00835231 (**5**) (Fig. 1), showed high in vitro potency against SARS-CoV-2 [18].

Lead discovery and optimization

The first-generation M^{pro} inhibitors, such as PF-07304814 (**6**) and GC-376 (**7**) (Fig. 1A), showed promising inhibition of SARS-CoV-2 in vitro, but they displayed poor oral bioavailability in rats [18]. For example, PF-00835231 (**5**) was reported to have 1.4% bioavailability and GC-376 (**7**) has 3% bioavailability in rats [18]. The highly water-soluble phosphate prodrug form of (**5**), PF-07304814 (**6**), was designed for intravenous administration and is currently undergoing trials to be utilized for hospitalized patients [16, 18]. These first-generation M^{pro} inhibitors provided promising starting points for the development of subsequent second-generation M^{pro} inhibitors.

The origin of the P1 pyrrolidone substituent

The P1 pyrrolidone in nirmatrelvir (**3**) remains unchanged from the lead molecule PF-00835231 (**5**), which is also present on the P1 position of the broad-spectrum antiviral GC-376 (**7**) [16, 23]. SARS-CoV-2 M^{pro} has a stringent substrate preference of glutamine in the P1 position [16–18]. As such, glutamine becomes an apparent choice for the P1 substituent in M^{pro} inhibitor. However, the issue with glutamine is that the side chain amide can react with the warhead to form the cyclized product (Fig. 1B) [24]. To circumvent this problem, cyclized pyrrolidone or 2-piperidinone were designed as a mimetic of the glutamine side chain [21]. The cyclic pyrrolidone provides rigidity over the higher flexibility of the glutamine side chain, likely providing a reduced loss of conformational entropy upon binding to the target [25]. The pyrrolidone moiety has been widely utilized in the design of rhinovirus inhibitor rupintrivir (**8**), GC-376 (**7**), and lead molecule PF-00835231 (**5**) [21, 22].

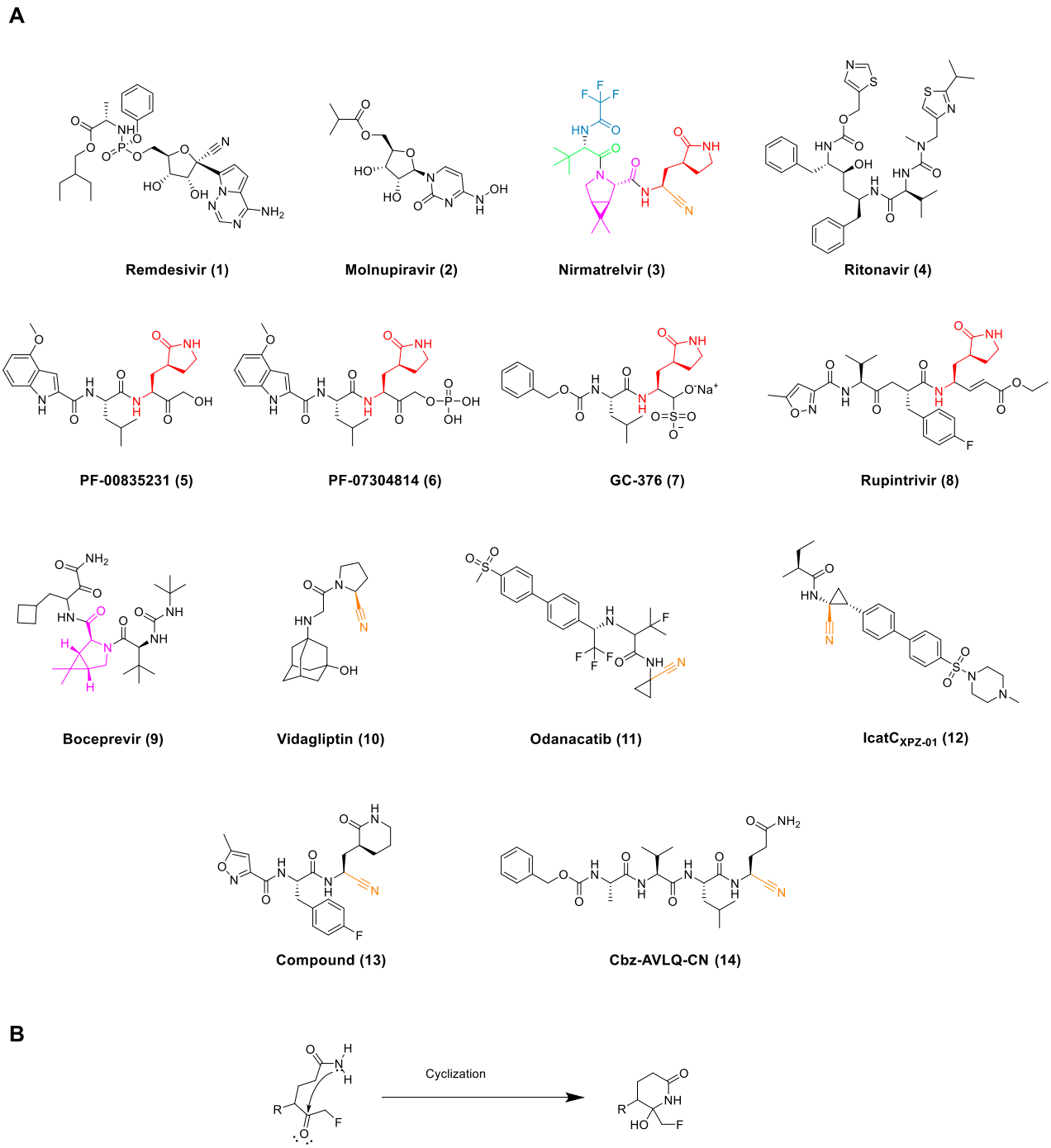


Fig. 1 Rational design of nirmatrelvir. **A** Chemical structures of remdesivir (**1**), molnupiravir (**2**), nirmatrelvir (**3**), ritonavir (**4**), PF-00835231 (**5**), PF-07304814 (**6**), GC-376 (**7**), rupintrivir (**8**), boceprevir (**9**), vidagliptin (**10**), odanacatib (**11**), IcatCXPZ-0 (**12**), compound **13**, and Cbz-AVLQ-CN (**14**). The nirmatrelvir substitutions, P, are labeled by color: the P1 group is shown in red, P1' in orange, P2 in pink, P3 in green, and P4 in blue; similar moieties present on nirmatrelvir (**3**) are colored on other molecules. **B** The cyclization between the glutamine sidechain amide with the warhead carbonyl [24]

The origin of the P2 6,6-dimethyl-3-azabicyclo[3.1.0] hexane substituent

The addition of a 6,6-dimethyl-3-azabicyclo[3.1.0]hexane at the P2 position removed the hydrogen bond doner between the P2/P3 amide linkage; this moiety is also present on the previously established HCV NS3/4A serine protease inhibitor boceprevir (**9**) [16, 23, 26]. Our group and others independently discovered boceprevir as a SARS-CoV-2 M^{pro} inhibitor [23, 27]. The X-ray crystal structure of SARS-CoV-2

M^{pro} in complex with boceprevir (**9**) provides a structural basis for the incorporation of 6,6-dimethyl-3-azabicyclo[3.1.0]hexane at the P2 position of nirmatrelvir (**3**) [27].

The origin of the nitrile reactive warhead

The nitrile is an established and pharmacologically compliant reactive warhead for targeting serine and cysteine proteases. The nitrile warhead has previously been explored as a reactive group in FDA-approved drugs such as the nitrile containing dipeptidyl peptidase-4 (DPP-4) inhibitor vildagliptin (**9**) [28]. The nitrile from the antidiabetic vildagliptin (**9**) covalently reacts with the catalytic serine of DPP-4, a serine protease that mediates the incretine levels [28].

In terms of targeting cysteine proteases, odanacatib (**11**) is a cathepsin K inhibitor that was advanced in clinical trial for the treatment of osteoporosis and bone metastasis [28–30]. The nitrile warhead has also been utilized in the cathepsin C inhibitor $IcatC_{XPZ\text{-}01}$ (**12**) [31, 32]. The nitrile warhead has also been explored for the viral cysteine proteases, such as compound (**13**) and Cbz-AVLQ-CN (**14**) for enterovirus A71 (EV-A71) $3CL^{pro}$ [29, 33, 34]. Nitrile warheads are comparatively inert when compared to other electrophiles, such as aldehydes, requiring highly reactive active site nucleophiles along with precise positioning of the electrophilic nitrile carbon atom, enhancing high target selectivity [28, 31, 35, 36]. The replacement of the hydrogen bond donor of the α-hydroxymethyl ketone covalent warhead in PF-00835231 (**5**) with a nitrile group compound (**15**) or benzothiazol-2-yl ketone compound (**16**) improved oral bioavailability (Table 1) [16, 33].

Lead optimization

The nitrile containing compound (**15**) (Table 1) had an increase in rat oral bioavailability (7.6%) and acceptable metabolic stability when exposed to human liver microsomes; however, compound (**15**) had a decrease in enzymatic inhibitory potency (K_i) and antiviral activity (EC_{50}) (Table 1) [16, 37]. To identify an optimal alternate P3 capping group, sulfonamide was replaced with trifluoroacetamide in compound (**18**) [16]. Compound (**18**) (Table 1) exhibited comparable biochemical potency to (**17**) (Table 1) but with significantly improved SARS-CoV-2 Vero E6 antiviral activity and further increased metabolic stability [16]. Replacing the benzothiazol-2-yl ketone warhead with a nitrile and replacing the isopropyl group with a tertbutyl at the P3 location on compound (**18**) led to the compound Nirmatrelvir (**3**), a second-generation M^{pro} inhibitor [16].

The nitrile compound, Nirmatrelvir (**3**), was selected over benzothiazol-2-yl ketone-containing compound (**18**) as the clinical candidate based on the perceived ability to more easily scale up synthesis, a reduced likelihood of epimerization at the P1 stereocenter, and better solubility (Table 1) [16].

Dosage, toxicity, metabolism, and selectivity

Through clinical trials, nirmatrelvir/ritonavir has been shown to reduce hospitalizations by 89% [9]. Nirmatrelvir (**3**) and ritonavir (**4**) are administered twice daily every 12 h in the form of three pills: two 150 mg pills of nirmatrelvir (**3**) and one 100 mg pill of ritonavir (**4**), the drugs are administered for five days with a total of 30 pills [7, 9, 11, 38]. The FDA cautions that this drug combination is not recommended for patients with severe liver or renal function impairment [11]. Established side effects include nausea, diarrhea, and increased blood pressure [11].

Preclinical evaluations of nirmatrelvir (**3**) in vitro showed promising off-target selectivity as it did not interfere with a broad range of G protein-coupled receptors, kinases, transporters, and other phosphodiesterase enzyme inhibitor screens [16]. It also showed no noticeable activity against the cardiac ion channels Kv1.1, Cav1.2, and Nav1.5 [16].

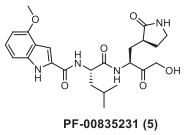
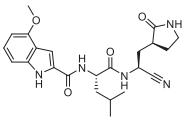
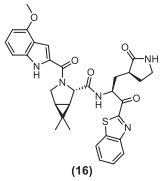
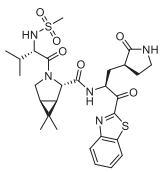
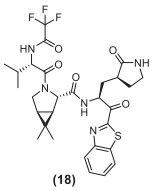
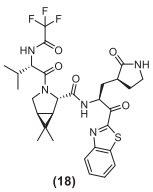
Nirmatrelvir (**3**) was tested for selectivity against host and HIV proteases; all tested proteases had IC_{50} values above 100 μM, suggesting a low potential for off-target effects (Table 2) [16]. In vivo testing showed nirmatrelvir (**3**) was readily metabolized by CYP3A4; this led to the conclusion that inhibiting CYP3A4 with the established CYP3A4 inhibitor ritonavir (**4**) would increase the therapeutic concentration of nirmatrelvir (**3**) [16]. Nirmatrelvir (**3**) did not display any mutagenic or clastogenic effects [16]. Ritonavir (**4**) has no inhibitory activity against M^{pro} nor antiviral activity against SARS-CoV-2 [39]. As stated prior, the EPIC-HR trial showed a significant decrease in hospitalizations related to COVID-19 in high-risk non-hospitalized patients, and an interim analysis by Pfizer of phase 2/3 showed a decrease in mortality and hospitalization [7].

As stated prior, ritonavir (**4**) is a potent cytochrome P450 (CYP) inhibitor with well-established toxicity secondary to potentially life-threatening drug-drug interactions [38]. The use of ritonavir (**4**) may require dose adjustments or discontinuation of conflicting drugs [38, 40].

Synthetic methods for the synthesis of nirmatrelvir

The main route for the synthesis of nirmatrelvir (**3**) was proposed by Owen et al. [16]. According to the pathway shown in Scheme 1, nirmatrelvir MTBE solvate was synthesized from the starting material methyl (1R,2S,5S)-6,6-dimethyl-3-azabicyclo [3.1.0]hexane-2-carboxylate, HCl salt in six steps [16]. Compound (**20**), used in the fourth step of Scheme 1A, is synthesized in two reactions [16]. The

Table 1 In vitro and in vivo parameters optimized in identifying oral SARS-CoV-2 M^{pro} inhibitors [16]

Structure	SARS-CoV-2 M^{pro} K_i (nM)	VeroE6-enACE2 CPE EC_{50} (nM)	HLM CL_{int} (µl/min/mg)	Mean Oral F (%)
 PF-00835231 (5)	0.271 (0.155–0.471, $n = 6$)	231 (158–338, $n = 8$)	7.47 ± 0.88	1.4 ± 0.8
 (15)	27.7 (18.4–41.7, $n = 5$)	1364 (860–2164, $n = 15$)	34.4 ± 0.7	7.6 (7.4, 7.8)
 (16)	230 (181–292, $n = 4$)	5593 (3457–9051, $n = 8$)	337 ± 9	N.D.
 (17)	7.93 (3.62–17.4, $n = 5$)	909 (557–1482, $n = 14$)	127 ± 3	10 (7.5, 13)
 (18)	12.1 (8.05–18.1, $n = 7$)	85.3 (76.5–95.2, $n = 36$)	30.3 ± 0.6	33 (33, 34)
 (18)	3.11 (1.47–6.59, $n = 6$)	74.5 (66.5–83.4, $n = 20$)	24.5 ± 0.2	50 (30, 71)

The K_i values are given for each compound in nM. EC_{50} values were calculated with assays using Vero E6 cells enriched for ACE2; data were normalized to controls and recorded in nM. The total intrinsic clearance (CL_{int}) was obtained from the scaling of half-lives of test compounds in NADPH-supplemented human liver microsomes (HLM) and was recorded in µl/min/mg. Rat oral absorption (Oral F) was recorded as a percentage N.D. means not determined [16]

nirmatrelvir, MTBE solvate can be purified via recrystallization to synthesize anhydrous nirmatrelvir (**3**) product [16].

The ester of starting material (**19**) is converted to a primary amide via aminolysis, following aminolysis the Boc protecting group is removed to generate compound (**20**) with a 96% yield [16].

The synthetic pathway starts with amide coupling of (**21**) and N-(tert- butoxycarbonyl)-3-methyl-L-valine to synthesize (**22**); ester hydrolysis is utilized to synthesize compound (**23**). The Boc protection group is then removed so the primary amine can react with ethyl trifluoroacetate to synthesize compound (**24**). Amide coupling is utilized to synthesize compound (**25**) from compounds (**20**) and (**24**). The primary amide is dehydrated to the nitrile warhead and nirmatrelvir (**3**) is synthesized as an MTBE solvate with a 49.6% yield. Recrystallization was then used to purify nirmatrelvir (**3**) and remove MTBE [16].

A second alternate two-step pathway was utilized by Zhau et al., which is considerably shorter than the scheme proposed by Owen et al. and relies primarily on amide coupling and ester hydrolysis [16, 41].

The proposed Scheme 1B begins with amide coupling using (**26**) and L-Valine, 3-methyl-*N*-(trifluoroacetyl) to synthesize intermediate (**27**). The ester on compound (**27**) is then

hydrolyzed, and amide coupling is utilized again the synthesize the final product Nirmatrelvir (**3**), with a 60% yield.

While the synthesis pathway reported by Zhau et al. was shorter than the scheme reported by Owen et al., it was utilized for a smaller scale synthesis of 39 mg compared to 160.9 g reported by Owen et al. [16, 41].

Crystal structure of nirmatrelvir-M^{pro} complex

To form the covalent bond between the inhibitor and C145, the catalytic dyad is established after an initial proton transfer from C145 to H41 [42]. The negatively charged C145 approaches the electrophilic nitrile of nirmatrelvir (**3**) [42].

Table 2 Selectivity of nirmatrelvir (**3**) against mammalian and HIV proteases

Protease	IC_{50} (μM)
Human Cathepsin B	>100
Bovine Chymotrypsin	>100
Human Thrombin	>100
Human Caspase 2	>100
Human Cathepsin D	>100
Human Cathepsin L	>100
Human Immunodeficiency Virus-1	>100
Human Elastase	>100

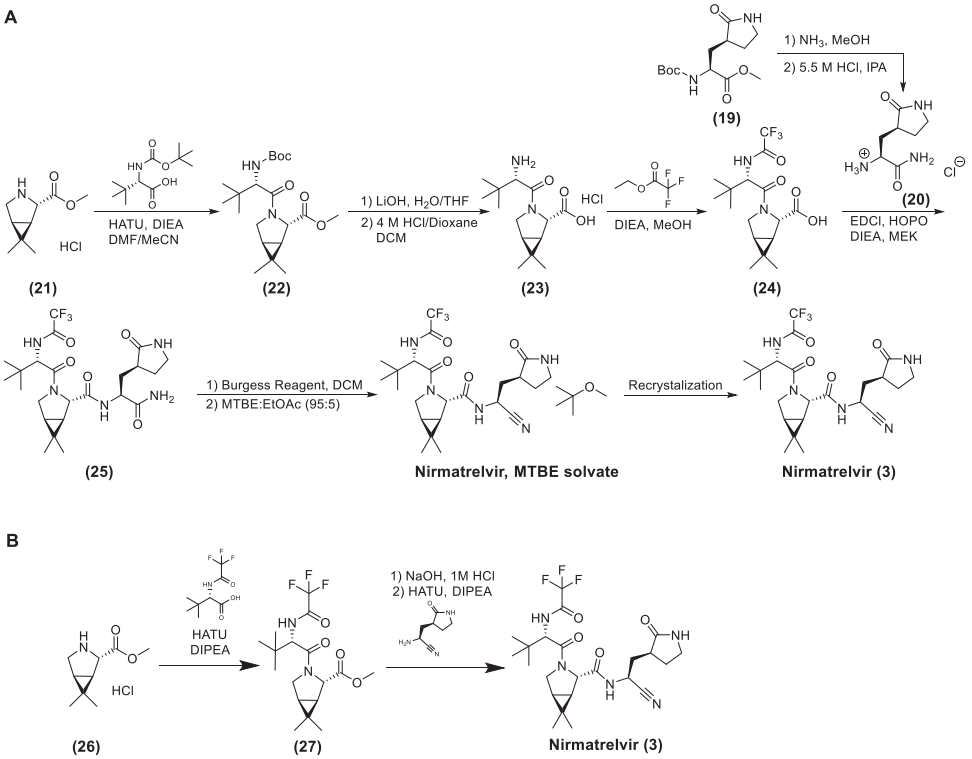
Data shown as IC_{50} values [15]

There are two transferred protons at the transition state: from H41 to a water molecule and from this to the nitrogen atom of the nirmatrelvir (**3**) nitrile group [42]. Following the water-mediated proton transfer, a thioimidate product is formed, with a Sγ–C distance of 1.8 Å (Fig. 2) [42].

The nitrile warhead's small size may be advantageous in forming the covalent complex as a water molecule can be closer positioned to the active site stabilizing the ion pair [42]. This stabilization may also lower the activation energy and increase the reaction rate [42].

The crystal structure of the drug-protein complex provides an in-depth analysis of the inhibitory mechanism of nirmatrelvir (**3**) [41]. An electron density map from the crystal structure indicated that the nitrile carbon of nirmatrelvir (**3**) is linked to the Sγ atom of C145 through a 1.8-Å C-S covalent bond (Fig. 3A); the electron density map was also used to conclude the existence of a reversible non-covalently bound state [41]. The imine nitrogen of the thioimidate moiety occupies the previously mentioned oxyanion loop due to the hydrogen bonds with the G143 and C145 residues (Fig. 3B) [41].

There are extensive hydrophobic interactions between the cyclopropyl moiety and the hydrophobic side chains of H41, M49, Y54, M165, and D189, and the main chains of D187 and R188 (Fig. 3B) [41]. The hydrophobic P3 tert-butyl group had limited interactions with M^{pro} as it was exposed to the solvent (Fig. 3A) [41]. The P4 trifluoroacetyl group folds into the S4 sub-pocket, possibly



Scheme 1 **A** A synthetic method for the synthesis of nirmatrelvir (**3**) developed by Pfizer. Two steps are utilized to generate the intermediate (**20**) with a 96% yield. The six-step synthesis has a total yield of 49.6% to synthesize the nirmatrelvir, MTBE solvate, and recrystallization is conducted to purify the solvate [16]. **B** The synthetic method for the synthesis of nirmatrelvir (**3**) was reported by Zhau et al. The two-step synthesis has a total yield of 60% [37]

due to the trifluoromethyl group forming hydrogen bonds with Q192 and ordered water molecules (Fig. 3A) [41]. The P4 amide nitrogen forms a hydrogen bond with the main-chain carbonyl oxygen of E166 (Fig. 3B) [41]. While the covalent bond between the nitrile carbon and the C145 sulfur is important for inhibition, the stabilization through a system of hydrogen bonds and hydrophobic interactions enhances its binding to the active site of M^{pro} [41].

Metabolism of nirmatrelvir

Metabolism of nirmatrelvir (**3**) occurs through oxidation by CYP3A4 [43]. The isolated metabolites were from the mono-hydroxylation (Fig. 4, **29**–**32**) and dehydrogenation (Fig. 4, **28**) of nirmatrelvir (**3**) [43]. As stated previously, ritonavir (**4**) was utilized to decrease the metabolism of nirmatrelvir (**3**) by CYP3A4 therefor increasing its half-life and making the drug more effective.

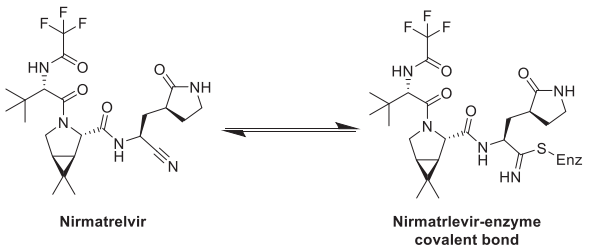


Fig. 2 Chemical structure of the nirmatrelvir (**3**) and the thioimidate complex formed after reversable covalent inhibition with the M^{pro} catalytic cysteine [42]

M^{pro} mutation and resistance

Emerging SARS-CoV-2 variants, such as the Omicron variant, have generated concern as there are multiple mutations to the spike protein that may potentially allow the virus to evade immune responses triggered by vaccines [44, 45]. This concern is also shared with antiviral resistance, although it was found that nirmatrelvir (**3**) potently inhibits the Omicron variant and reduces viral titers [45]. The most prevalent M^{pro} missense mutations (G15S, T21I, L89F, K90R, P132H, L205V, A260V in Fig. 5) discovered in various SARS-CoV-2 lineages (C.37 Lambda, B.1.1.318, B.1.2, B.1.351 Beta, B.1.1.529 Omicron, P.2 Zeta) do not convey antiviral resistance as the viral main protease is still susceptible to nirmatrelvir (**3**) [46, 47].

Perspective

While the main target of nirmatrelvir (**3**) is SARS-CoV-2 M^{pro}, nirmatrelvir (**3**) has shown broad-spectrum inhibitory activity against other known human coronavirus main proteases (Table 3), including beta-coronaviruses (SARS-CoV-2, SARS-CoV-1, MERS-CoV, HKU1, and OC43) and alpha-coronaviruses (229E and NL63) [16]. However, the potential broad-spectrum activity of nirmatrelvir (**3**) has not yet been investigated in vivo [16] and it might be worthwhile to test the efficacy of nirmatrelvir in related animal models. In addition, direct-acting combination therapies may provide more effective treatment of COVID-19. When in combination with **6**, synergistic antiviral activity was observed against the Omicron variant in vitro [45]. Further investigation of synergistic drug

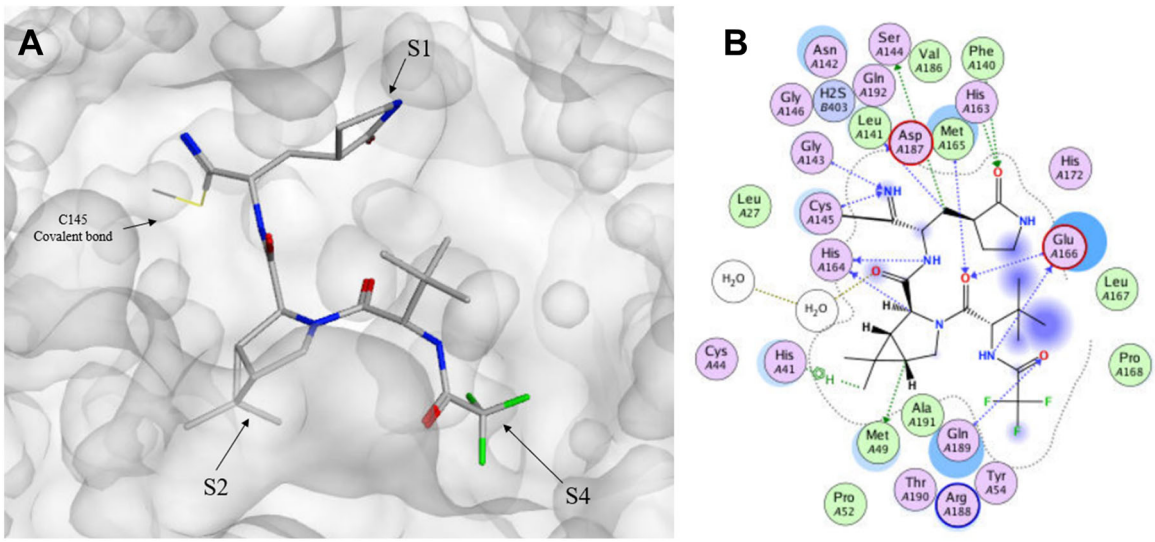


Fig. 3 **A** The zoom-in view of the substrate-binding pocket. **B** Diagram of the interactions between nirmatrelvir (**3**) and SARS-CoV-2 M^{pro} generated in MOE [41]

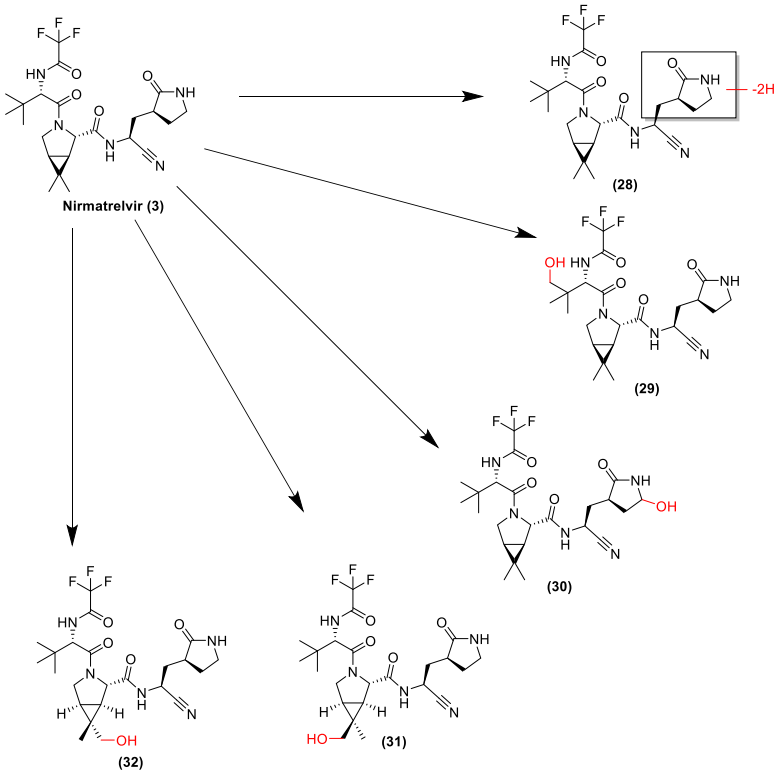


Fig. 4 Metabolism of nirmatrelvir (**3**) via liver microsomes and hepatocytes from rat, monkey, and human

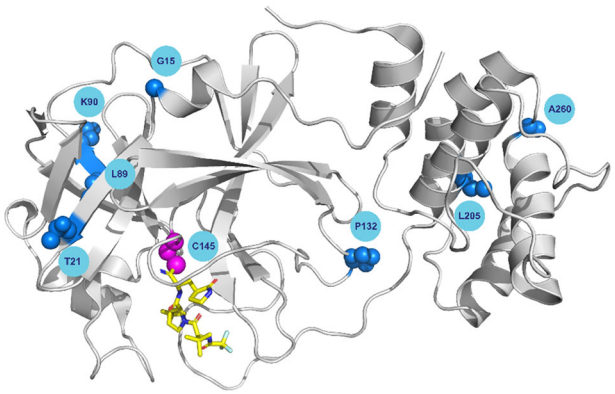


Fig. 5 SARS-CoV-2 M^{pro} mutants. High frequency M^{pro} mutants are mapped to the X-ray crystal structure of SARS-CoV-2 M^{pro} in complex with nirmatrelvir (PDB: 7SI9). Nirmatrelvir (**3**) is shown in sticks and colored in yellow. The catalytic C145 is shown in spheres and colored in magenta. The high frequency mutant residues G15S, T21I, L89F, K90R, P132H, L205V, and A260V are shown in spheres and colored in marine

Table 3 Nirmatrelvir (**3**) inhibitory effect on other human coronaviruses main proteases

Virus main protease	Nirmatrelvir K_i (nM)
SARS-CoV-2 M^{pro}	3.11 (1.31–4.91)
SARS-CoV-1 M^{pro}	4.94 (1.71–19.1)
NL63-CoV M^{pro}	226 (95.7–535)
229E-CoV M^{pro}	44.4 (10.4–189)
MERS-CoV M^{pro}	187 (166–211)
HKU1-CoV M^{pro}	189 (135–263)
OC43-CoV M^{pro}	36.4 (8.92–149)

Data shown as inhibitory constants (K_i) in nM relative to no inhibitor controls and complete inhibition with 30 μM 5 [16].

combinations should be explored to provide higher quality treatment of COVID-19 and decrease the likelihood of acquired drug resistance.

Despite the efficacy of nirmatrelvir (**3**), P3 and P4 have a much smaller free energy contribution to the binding process with the target than P1 and P2, suggesting there may be potential to improve drug binding affinity to M^{pro} [42]. Further improving the binding affinity could further improve potency and selectivity. The improvement of the metabolic stability of nirmatrelvir (**3**) may be of interest to remove the reliance on coadministration with ritonavir (**4**).

Conclusion

Following the rapid spread of COVID-19, the waves of new variants, and the resultant devastation, there is an increasing need for the fast-track development of cost-effective antivirals for outpatient use. Researchers in many fields have contributed to developing and evaluating small molecule therapeutics for this purpose. With the quick development of nirmatrelvir (**3**) and its emergency

authorization, this drug could prove to have a substantial impact in preventing COVID-19 related hospitalizations and deaths.

Acknowledgements This research was supported by the National Institutes of Health (NIH) (grants AI147325, AI157046, and AI158775) to JW.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

1. Grubaugh ND, Hodcroft EB, Fauver JR, Phelan AL, Cevik M. Public health actions to control new SARS-CoV-2 variants Comment. Cell. 2021;184:1127–32. https://doi.org/10.1016/j.cell.2021.01.044.
2. Johns Hopkins Coronavirus Resource Center. 2022. https://coronavirus-jhu-edu.proxy.libraries.rutgers.edu/map.html.
3. Fernandes Q, Inchakalody VP, Merhi M, Mestiri S, Taib N, El-Ella DMA, et al. Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. Ann Med. 2022;54:524–40. https://doi.org/10.1080/07853890.2022.2031274.
4. Kontoghiorghes GJ, Fetta S, Kontoghiorghe CN. The need for a multi-level drug targeting strategy to curb the COVID-19 pandemic. Front Biosci. 2021;26:1723–36. https://doi.org/10.52586/5064.
5. Schooley RT, Carlin AF, Beadle JR, Valiaeva N, Zhang XQ, Clark AE, et al. Rethinking remdesivir: synthesis, antiviral activity, and pharmacokinetics of oral lipid prodrugs. Antimicrob Agents Chemother. 2021;65. https://doi.org/10.1128/aac.01155-21.
6. Yan V, Muller F. Comprehensive summary supporting clinical investigation of GS-441524 for Covid-19 treatment. 2020. https://doi.org/10.31219/osf.io/mnhxu.
7. Parums DV. Editorial: Current status of oral antiviral drug treatments for SARS-CoV-2 infection in non-hospitalized patients. Med Sci Monit. 2022;28. https://doi.org/10.12659/msm.935952.
8. Bethany H. To conquer COVID-19, create the perfect pill. Chem Eng N. 2021;99:28–31. https://doi.org/10.47287/cen-09919-cover1.
9. Ritonavir-Boosted Nirmatrelvir (Paxlovid). National Institutes of Health, COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. 2022. https://www.covid19treatmentguidelines.nih.gov/therapies/antiviral-therapy/ritonavir-boosted-nirmatrelvir--paxlovid-/.
10. Hammond J, Leister-Tebbe H, Gardner A, Abreu P, Bao W, Wisemandle W, et al. Oral nirmatrelvir for high-risk, nonhospitalized adults with COVID-19. N Engl J Med. 2022;386:1397–408. https://doi.org/10.1056/NEJMoa2118542.
11. Food and Druga Administration (FDA) Emergency use authorization 105. Paxlovid (nirmatrelvir co-packaged with ritonavir) for the treatment of mild-to-moderate coronavirus disease 2019 (COVID-19) in certain adults and pediatric patients. 2021. https://www.fda.gov/media/155049/download.
12. Heskin J, Pallett SJC, Mughal N, Davies GW, Moore LSP, Rayment M, et al. Caution required with use of ritonavir-boosted PF-07321332 in COVID-19 management. Lancet. 2022;399:21–2. https://doi.org/10.1016/S0140-6736(21)02657-X.
13. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579:265–9. https://doi.org/10.1038/s41586-020-2008-3.
14. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature. 2020;582:289–93. https://doi.org/10.1038/s41586-020-2223-y.
15. Pillaiyar T, Manickam M, Namasivayam V, Hayashi Y, Jung S-H. An Overview of Severe Acute Respiratory Syndrome–Coronavirus (SARS-CoV) 3CL protease inhibitors: peptidomimetics and small molecule chemotherapy. J Med Chem. 2016;59:6595–628. https://doi.org/10.1021/acs.jmedchem.5b01461.
16. Owen DR, Allerton CMN, Anderson AS, Aschenbrenner L, Avery M, Berritt S, et al. An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. Science. 2021;374:1586–93. https://doi.org/10.1126/science.abl4784.
17. Fan KQ, Ma L, Han XF, Liang HH, Wei P, Liu Y, et al. The substrate specificity of SARS coronavirus 3C-like proteinase. Biochem Biophys Res Commun. 2005;329:934–40. https://doi.org/10.1016/j.bbrc.2005.02.061.
18. Vandyck K, Deval J. Considerations for the discovery and development of 3-chymotrypsin-like cysteine protease inhibitors targeting SARS-CoV-2 infection. Curr Opin Virol. 2021;49:36–40. https://doi.org/10.1016/j.coviro.2021.04.006.
19. Macchiagodena M, Pagliai M, Procacci P. Characterization of the non-covalent interaction between the PF-07321332 inhibitor and the SARS-CoV-2 main protease. J Mol Graph Model. 2022;110:108042. https://doi.org/10.1016/j.jmgm.2021.108042.
20. Ullrich S, Nitsche C. The SARS-CoV-2 main protease as drug target. Bioorg Med Chem Lett. 2020;30:127377. https://doi.org/10.1016/j.bmcl.2020.127377.
21. Cheng S-C, Chang G-G, Chou C-Y. Mutation of Glu-166 blocks the substrate-induced dimerization of SARS coronavirus main protease. Biophys J. 2010;98:1327–36. https://doi.org/10.1016/j.bpj.2009.12.4272.
22. Rut W, Groborz K, Zhang L, Sun X, Zmudzinski M, Pawlik B, et al. SARS-CoV-2 Mpro inhibitors and activity-based probes for patient-sample imaging. Nat Chem Biol. 2021;17:222–8. https://doi.org/10.1038/s41589-020-00689-z.
23. Ma CL, Sacco MD, Hurst B, Townsend JA, Hu YM, Szeto T, et al. Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease. Cell Res. 2020;30:678–92. https://doi.org/10.1038/s41422-020-0356-z.
24. Zhang H-Z, Zhang H, Kemnitzer W, Tseng B, Cinatl J, Michaelis M, et al. Design and synthesis of dipeptidyl glutaminyl fluoromethyl ketones as potent Severe Acute Respiratory Syndrome Coronovirus (SARS-CoV) inhibitors. J Med Chem. 2006;49:1198–201. https://doi.org/10.1021/jm0507678.
25. Tan J, George S, Kusov Y, Perbandt M, Anemüller S, Mesters JR, et al. 3C protease of enterovirus 68: structure-based design of Michael acceptor inhibitors and their broad-spectrum antiviral effects against picornaviruses. J Virol. 2013;87:4339–51. https://doi.org/10.1128/JVI.01123-12.
26. Venkatraman S. Discovery of boceprevir, a direct-acting NS3/4A protease inhibitor for treatment of chronic hepatitis C infections. Trends Pharm Sci. 2012;33:289–94. https://doi.org/10.1016/j.tips.2012.03.012.
27. Fu L, Ye F, Feng Y, Yu F, Wang Q, Wu Y, et al. Both Boceprevir and GC376 efficaciously inhibit SARS-CoV-2 by targeting its main protease. Nat Commun. 2020;11:4417. https://doi.org/10.1038/s41467-020-18233-x.
28. Brogi S, Ibba R, Rossi S, Butini S, Calderone V, Gemma S, et al. Covalent reversible inhibitors of cysteine proteases containing the nitrile warhead: recent advancement in the field of viral and parasitic diseases. Molecules. 2022;27. https://doi.org/10.3390/molecules27082561.
29. Bai B, Arutyunova E, Khan MB, Lu J, Joyce MA, Saffran HA, et al. Peptidomimetic nitrile warheads as SARS-CoV-2 3CL protease inhibitors. RSC Med Chem. 2021;12:1722–30. https://doi.org/10.1039/D1MD00247C.
30. Ndao M, Beaulieu C, Black WC, Isabel E, Vasquez-Camargo F, Nath-Chowdhury M, et al. Reversible cysteine protease inhibitors

show promise for a chagas disease cure. Antimicrob Agents Chemther. 2014;58:1167–78. https://doi.org/10.1128/AAC.01855-13.
31. Silva DG, Ribeiro JFR, De Vita D, Cianni L, Franco CH, Freitas-Junior LH, et al. A comparative study of warheads for design of cysteine protease inhibitors. Bioorg Med Chem Lett. 2017;27:5031–5. https://doi.org/10.1016/j.bmcl.2017.10.002.
32. Korkmaz B, Lesner A, Wysocka M, Gieldon A, Hakansson M, Gauthier F, et al. Structure-based design and in vivo anti-arthritic activity evaluation of a potent dipeptidyl cyclopropyl nitrile inhibitor of cathepsin C. Biochem Pharm. 2019;164:349–67. https://doi.org/10.1016/j.bcp.2019.04.006.
33. Chuck C-P, Chen C, Ke Z, Chi-Cheong Wan D, Chow H-F, Wong K-B. Design, synthesis and crystallographic analysis of nitrile-based broad-spectrum peptidomimetic inhibitors for coronavirus 3C-like proteases. Eur J Med Chem. 2013;59:1–6. https://doi.org/10.1016/j.ejmech.2012.10.053.
34. Zhai Y, Zhao X, Cui Z, Wang M, Wang Y, Li L, et al. Cyanohydrin as an anchoring group for potent and selective inhibitors of enterovirus 71 3C protease. J Med Chem. 2015;58:9414–20. https://doi.org/10.1021/acs.jmedchem.5b01013.
35. Gehringer M, Laufer SA. Emerging and re-emerging warheads for targeted covalent inhibitors: applications in medicinal chemistry and chemical biology. J Med Chem. 2019;62:5673–724. https://doi.org/10.1021/acs.jmedchem.8b01153.
36. Martin JS, MacKenzie CJ, Fletcher D, Gilbert IH. Characterising covalent warhead reactivity. Bioorg Med Chem. 2019;27:2066–74. https://doi.org/10.1016/j.bmc.2019.04.002.
37. Kato M, Chiba K, Hisaka A, Ishigami M, Kayama M, Mizuno N, et al. The intestinal first-pass metabolism of substrates of CYP3A4 and P-glycoprotei—quantitative analysis based on information from the literature. Drug Metab Pharmacokinet. 2003;18:365–72. https://doi.org/10.2133/dmpk.18.365.
38. Lange NW, Salerno DM, Jennings DL, Choe J, Hedvat J, Kovac D, et al. Nirmatrelvir/ritonavir use: managing clinically significant drug-drug interactions with transplant immunosuppressants. Am J Transplant. 2022. https://doi.org/10.1111/ajt.16955.
39. Ma C, Tan H, Choza J, Wang Y, Wang J. Validation and invalidation of SARS-CoV-2 main protease inhibitors using the Flip-GFP and Protease-Glo luciferase assays. Acta Pharm Sin B. 2022;12:1636–51. https://doi.org/10.1016/j.apsb.2021.10.026.
40. Hollywood P, MacCann R, Lorigan D, de Barra E, McConkey S. Pharmacokinetic enhancers (cobicistat/ritonavir) and the potential for drug-drug interactions. Ir J Med Sci. 2020;189:693–9. https://doi.org/10.1007/s11845-019-02125-1.
41. Zhao Y, Fang C, Zhang Q, Zhang RX, Zhao XB, Duan YK, et al. Crystal structure of SARS-CoV-2 main protease in complex with protease inhibitor PF-07321332. Protein Cell. https://doi.org/10.1007/s13238-021-00883-2.
42. Ramos-Guzman CA, Ruiz-Pernia JJ, Tunon I. Computational simulations on the binding and reactivity of a nitrile inhibitor of the SARS-CoV-2 main protease. Chem Commun. 2021;57:9096–9. https://doi.org/10.1039/d1cc03953a.
43. Eng H, Dantonio AL, Kadar EP, Obach RS, Di L, Lin J, et al. Disposition of PF-07321332 (Nirmatrelvir), an orally bioavailable inhibitor of SARS-CoV-2 3CL protease, across animals and humans. Drug Metab Dispos. 2022;50:576–90. https://doi.org/10.1124/dmd.121.000801.
44. Callaway E. Heavily mutated Omicron variant puts scientists on alert. Nature. 2021;600:21. https://doi.org/10.1038/d41586-021-03552-w.
45. Li PF, Wang YN, Lavrijsen M, Lamers MM, de Vries AC, Rottier RJ, et al. SARS-CoV-2 Omicron variant is highly sensitive to molnupiravir, nirmatrelvir, and the combination. Cell Res. 2022;32:322–4. https://doi.org/10.1038/s41422-022-00618-w.
46. Ullrich S, Ekanayake KB, Otting G, Nitsche C. Main protease mutants of SARS-CoV-2 variants remain susceptible to nirmatrelvir. Bioorg Med Chem Lett. 2022;62:128629. https://doi.org/10.1016/j.bmcl.2022.128629.
47. Hung Y-P, Lee J-C, Chiu C-W, Lee C-C, Tsai P-J, Hsu IL, et al. Oral nirmatrelvir/ritonavir therapy for COVID-19: the dawn in the dark? Antibiotics. 2022;11. https://doi.org/10.3390/antibiotics11020220.