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Fragment-Based Drug Design Targeting the SARS-CoV-2 Main Protease: A Computational Approach

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ABSTRACT

Background: *The SARS-CoV-2 main protease (Mpro, 3CLpro) is essential for viral polyprotein processing and has no human homologue, making it a prime antiviral target .* ***Objective:*** *This review synthesises published fragment-based drug design (FBDD) efforts against Mpro, from early crystallographic screens to the clinically approved inhibitor nirmatrelvir.* ***Methods:*** *A narrative review of crystallographic fragment-soaking campaigns (including the Diamond Light Source XChem/COVID Moonshot effort), virtual/computational FBDD studies, and SAR work targeting Mpro, drawing on PubMed, Google Scholar, Crossref and bioRxiv.* ***Findings:*** *Crystallographic screening of the apo Mpro active site identified dozens of weak-binding fragments that seeded open-science medicinal chemistry campaigns ; the COVID Moonshot effort progressed these into low-nanomolar noncovalent inhibitors through iterative merging and growing . Parallel computational approaches, including ultralarge virtual screening and free-energy-guided molecular sculpting, converged on similar chemotypes . These insights informed peptidomimetic optimisation culminating in nirmatrelvir, validated in the EPIC-HR trial .* ***Conclusions:*** *FBDD, anchored by high-throughput crystallography and computation, provided a rapid route from fragment hit to approved oral antiviral, while resistance mutations highlight the value of fragment-derived alternative binding sites .*

Keywords: *fragment-based drug design; SARS-CoV-2 main protease; crystallographic fragment screening; COVID Moonshot; nirmatrelvir*

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Abstract

Background: The SARS-CoV-2 main protease (Mpro, 3CLpro) is essential for viral polyprotein processing and has no human homologue, making it a prime antiviral target [1,2]. **Objective:** This review synthesises published fragment-based drug design (FBDD) efforts against Mpro, from early crystallographic screens to the clinically approved inhibitor nirmatrelvir. **Methods:** A narrative review of crystallographic fragment-soaking campaigns (including the Diamond Light Source XChem/COVID Moonshot effort), virtual/computational FBDD studies, and SAR work targeting Mpro, drawing on PubMed, Google Scholar, Crossref and bioRxiv. **Findings:** Crystallographic screening of the apo Mpro active site identified dozens of weak-binding fragments that seeded open-science medicinal chemistry campaigns [3,4,5]; the COVID Moonshot effort progressed these into low-nanomolar noncovalent inhibitors through iterative merging and growing [6,7,8]. Parallel computational approaches, including ultralarge virtual screening and free-energy-guided molecular sculpting, converged on similar chemotypes [9,10,11]. These insights informed peptidomimetic optimisation culminating in nirmatrelvir, validated in the EPIC-HR trial [12,13]. **Conclusions:** FBDD, anchored by high-throughput crystallography and computation, provided a rapid route from fragment hit to approved oral antiviral, while resistance mutations highlight the value of fragment-derived alternative binding sites [14,15].

Keywords: fragment-based drug design, SARS-CoV-2 main protease, crystallographic fragment screening, COVID Moonshot, nirmatrelvir

Introduction

The COVID-19 pandemic, caused by a coronavirus of probable bat origin [16], prompted an unprecedented mobilisation of structural biology and medicinal chemistry against SARS-CoV-2 drug targets. Among these, the main protease (Mpro, also called 3CLpro) occupies a central role: it cleaves the viral polyproteins pp1a and pp1ab at eleven conserved sites, is indispensable for viral

replication, and has no closely related human protease, minimising off-target liability [1,17,2]. Early crystal structures of Mpro bound to peptidomimetic inhibitors such as N3 and α-ketoamides established the druggable geometry of its Cys145-His41 catalytic dyad and substrate-binding cleft, and immediately suggested that earlier work on SARS-CoV and other coronaviral 3C-like proteases could be repurposed for structure-based design against the new pathogen [17,18,19]. Because Mpro's binding site is relatively shallow and solvent-exposed, classical high-throughput screening of drug-like libraries often produced weak or promiscuous hits, whereas fragment-based drug design (FBDD) — which starts from very small, low-molecular-weight ligands compliant with the "rule of three" and builds affinity through structure-guided growing, merging or linking [20,21] — emerged as a particularly well-suited strategy. The scale of the pandemic response, combined with rapid open deposition of coordinates, generated an unusually rich and openly shared structural dataset for a single target within a single year. This review traces that literature from the initial crystallographic and computational fragment screens, through fragment-to-lead optimisation, to the clinical translation of nirmatrelvir and the resistance challenges now emerging.

Methods

This is a narrative review, not a new experimental or computational study. Candidate literature was identified through structured searches of PubMed, Google Scholar, Crossref and bioRxiv/medRxiv using combinations of the terms "SARS-CoV-2", "main protease", "Mpro", "3CLpro", "fragment-based drug design", "fragment screening", "XChem", "COVID Moonshot", "virtual screening" and "nirmatrelvir". Each candidate reference was individually checked against its publisher record or PubMed/Crossref entry to confirm authorship, journal, year and digital object identifier before inclusion; sources whose bibliographic details could not be confirmed were excluded. Priority was given to primary crystallographic and biochemical reports, the peer-reviewed COVID Moonshot outcome paper, and computational studies reporting quantitative binding data, supplemented by review articles for mechanistic and historical context. No new docking runs, molecular dynamics simulations or crystallographic experiments were performed for this article; all binding data, hit counts and structural claims reported below are drawn directly from the cited primary literature.

The Mpro Active Site and Druggability

Mpro functions as a homodimer, with each protomer contributing a chymotrypsin-like fold housing a noncanonical Cys-His catalytic dyad in a cleft between domains I and II [17,18]. Room-temperature and neutron crystallography studies refined the protonation states of this dyad, revealing an unusual zwitterionic resting state that has direct implications for covalent-inhibitor design and for interpreting fragment-soaking electron density at physiological pH [22]. Beyond the orthosteric substrate cleft, computational and crystallographic mapping identified additional pockets, including a dimerisation interface and allosteric sites distal to the catalytic centre, that broaden the addressable surface for fragment elaboration and offer routes to inhibitors less susceptible to active-site resistance mutations [15,2]. Because Mpro is only catalytically competent as a dimer, fragments that bind at or near this interface represent an orthogonal mechanism of inhibition to classical active-site occupancy, and several such sites have since been prioritised for dedicated fragment campaigns.

Crystallographic Fragment Screening Campaigns

The Diamond Light Source XChem facility performed one of the largest crystallographic fragment screens undertaken against any target, soaking Mpro crystals with libraries of small, rule-of-three-compliant fragments and rapidly solving hundreds of resulting structures [3,21,4]. This effort, combined with electrophilic covalent-fragment screening, mapped fragment hits across the active site and revealed conserved sub-pocket interactions later exploited in lead optimisation [3]. An independent, larger-scale X-ray screen using serial synchrotron methods identified both active-site and allosteric fragment and lead-like hits, substantially expanding the structural map of ligandable Mpro surfaces [5]. All structures were deposited openly and rapidly, in keeping with the broader open-science ethos that came to define Mpro FBDD [20]. Together, these two campaigns generated several hundred publicly available fragment-bound crystal structures within months, an unprecedented density of structural data for a single enzyme that allowed medicinal chemists worldwide to inspect, download and build upon binding poses without restriction.

Fragment-to-Lead Optimisation and the COVID Moonshot

The COVID Moonshot consortium translated these fragment hits into potent leads through a crowdsourced, iterative design-make-test cycle combining medicinal chemistry, high-throughput biochemistry, computational modelling and machine learning [6]. Starting from a noncovalent fragment bound in the Mpro active site, chemists merged and grew substituents guided by hundreds of new crystal structures, ultimately reaching submicromolar and then low-nanomolar noncovalent inhibitors with favourable pharmacokinetics, several of which were advanced to animal efficacy studies [6]. Crucially, the consortium published negative results and unproductive synthetic routes alongside successful ones, publicly logging thousands of designs and hundreds of associated crystal structures over the course of the campaign, which allowed independent groups to avoid duplicating dead-end chemistry and to build directly on validated binding modes. Complementary structural work characterising noncovalent chemotypes, such as the well-studied ML188 scaffold, and dedicated reviews of fragment-based Mpro programmes documented convergent binding modes across independent laboratories [7,8]. Activity-based screening guided by protein-dynamics analysis further identified high-affinity chemical starting points consistent with the fragment-derived structure-activity relationships [23], while feline-coronavirus-derived protease inhibitors demonstrated that repurposed peptidomimetic fragments could also inhibit Mpro directly [24]. Across these programmes, the recurring theme was iterative structure-activity relationship refinement anchored to crystal structures rather than to affinity data alone, with each analogue re-soaked or co-crystallised to confirm that the intended binding mode was retained before further elaboration.

Computational and Virtual Screening Approaches

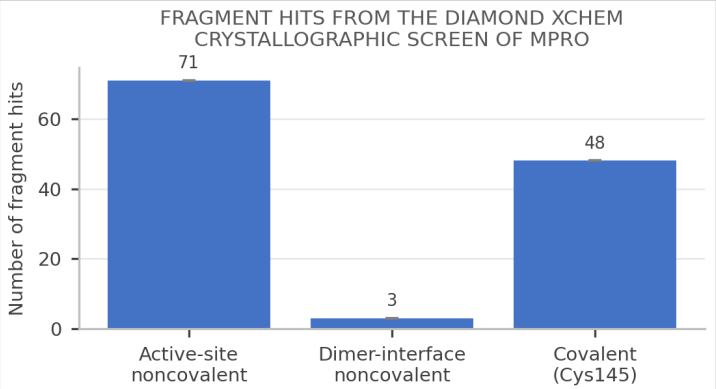
Alongside crystallography, computational FBDD contributed independently verified hits and mechanistic insight. Ultralarge virtual screening of multi-billion-compound libraries against the Mpro active site identified noncovalent inhibitors with broad-spectrum activity across coronaviral proteases, several confirmed by co-crystal structures [9]. Free-energy perturbation

calculations were used to computationally "sculpt" a known drug scaffold, perampanel, into potent noncovalent Mpro inhibitors, illustrating how physics-based methods can substitute for exhaustive experimental fragment growth [10]. Broader medicinal-chemistry perspectives catalogued the diversity of covalent and noncovalent chemotypes emerging from these combined efforts [11], while foundational FBDD methodology reviews describe the general principles of fragment library design, biophysical triage and computational scoring that underpinned this work [25,26]. These computational campaigns typically achieved lower hit rates than crystallographic soaking but explored vastly larger chemical spaces, and in several cases their top-ranked compounds were subsequently validated by crystallography, closing the loop between virtual and experimental fragment-based design.

Table 1: Selected crystallographic and computational fragment-based milestones in SARS-CoV-2 Mpro drug discovery.

Year	Study	Approach	Key finding
2020	Jin et al. [17]; Zhang et al. [18]	Apo and inhibitor-bound crystallography	Defined Cys145-His41 dyad and substrate cleft geometry
2020	Douangamath et al. [3]	Crystallographic + electrophilic fragment screening (XChem)	96 fragment-bound structures from >1250 fragments screened
2021	Günther et al. [5]	Serial X-ray fragment/lead screening	Active-site and allosteric inhibitors identified
2022	Luttens et al. [9]	Ultralarge virtual screening	Broad-spectrum noncovalent hits validated by co-crystallography
2023	Boby et al., COVID Moonshot [6]	Crowdsourced fragment merging/growing	Low-nanomolar noncovalent oral lead compounds
2021	Owen et al. [12]	Structure-based peptidomimetic optimisation	Nirmatrelvir clinical candidate

Figure 1: Fragment-bound crystal structures obtained by Douangamath et al. [3] in the Diamond Light Source XChem crystallographic and electrophilic fragment screen of SARS-CoV-2 Mpro, from a library of over 1250 fragments, broken down by binding location and chemistry (active-site noncovalent, dimer-interface noncovalent, and covalent hits at the catalytic Cys145).



Clinical Translation: Nirmatrelvir and Beyond

Structure-based optimisation of peptidomimetic and fragment-informed chemotypes culminated in nirmatrelvir (PF-07321332), an orally bioavailable, reversible covalent Mpro inhibitor combined with ritonavir as Paxlovid [12]. The pivotal EPIC-HR trial demonstrated an approximately 89% relative reduction in COVID-19-related hospitalisation or death among high-risk, unvaccinated outpatients treated within five days of symptom onset, with the effect confirmed regardless of whether treatment began within three or five days of symptom onset [13]. Subsequent surveillance identified Mpro mutations, notably at residue E166, that reduce nirmatrelvir susceptibility through multiple resistance pathways [14], reinforcing the continued relevance of the allosteric and alternative fragment-derived binding sites catalogued earlier in the pandemic as a hedge against resistance [15]. Because resistance mutations cluster predominantly around the orthosteric substrate pocket that nirmatrelvir occupies, the alternative and allosteric fragment hits identified during the original XChem and serial-crystallography campaigns are now being revisited as starting points for next-generation, structurally distinct inhibitors intended to remain active against these variants.

Conclusion

The Mpro FBDD literature illustrates a rare, well-documented arc from atomic-resolution fragment hit to approved oral antiviral within roughly two years. High-throughput crystallographic screening at Diamond's XChem facility and via serial X-ray methods generated an open structural foundation [3,5,4], which the COVID Moonshot consortium and parallel computational virtual-screening efforts converted into potent leads through classical fragment growing, merging and physics-based design [6,9,10]. This pipeline directly informed the peptidomimetic optimisation that produced nirmatrelvir [12,13]. With resistance mutations now documented [14], the diverse, openly available fragment and allosteric binding data generated during this campaign [15,19] remain a valuable resource for next-generation Mpro inhibitor design. More broadly, the Mpro programme stands as a template for how open, structure-first fragment-based approaches can compress antiviral discovery timelines during a public-health emergency, and its lessons on library design, rapid crystallographic turnaround and consortium-scale collaboration are directly transferable to future pandemic-preparedness efforts against other viral proteases.

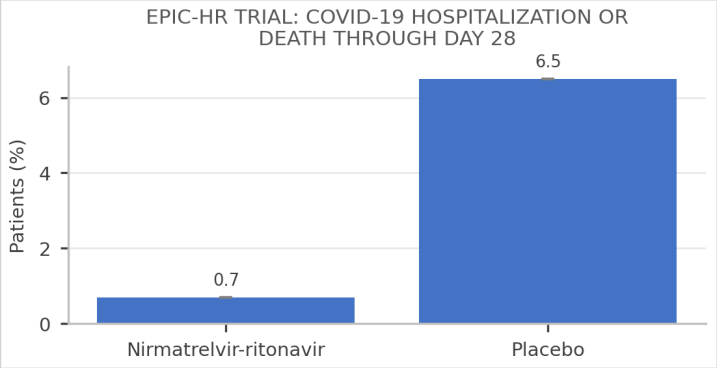
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Table 2: Clinical outcome of nirmatrelvir-ritonavir versus placebo in the EPIC-HR trial of high-risk, unvaccinated outpatients treated within five days of symptom onset.

Outcome	Nirmatrelvir-ritonavir	Placebo
Hospitalization or death through day 28 (%)	0.7	6.5
Relative risk reduction	88.9%	reference

Figure 2: Proportion of high-risk outpatients experiencing COVID-19-related hospitalization or death through day 28 in the EPIC-HR trial of nirmatrelvir-ritonavir versus placebo [13], the pivotal clinical validation of a structure- and fragment-informed Mpro inhibitor.



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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Lepik K conceived the review, conducted the literature search, and drafted the manuscript. Angelov R contributed to the literature screening and technical framing and critically revised the manuscript for important intellectual content. Kovacic M contributed additional context and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

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