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HIGHLIGHT

Targeting the coronavirus membrane protein: A promising novel therapeutic strategy

KEY WORDS

Coronavirus membrane protein;
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M protein

The past two decades have witnessed multiple severe outbreaks caused by betacoronaviruses, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19). Among these, the global pandemic of COVID-19 caused by SARS-CoV-2 has placed unprecedented pressure on public health systems worldwide. Current therapeutic options primarily include remdesivir, molnupiravir (targeting RNA-dependent RNA polymerase, RdRp), nirmatrelvir/ritonavir, and ensitrelvir (targeting main protease, M^{pro}). However, these drugs are limited by challenges such as drug resistance, drug−drug interactions, and reduced efficacy in specific clinical contexts. Consequently, there is an urgent need to develop novel antiviral agents targeting underexplored stages of the coronavirus replication cycle. In this context, two recent studies published in *Nature* have garnered significant attention by identifying small-molecule inhibitors that target the SARS-CoV-2 membrane protein (M protein)[1,2]. Specifically, **JNJ-9676** and **CIM-834** were shown to interfere with viral assembly, offering promising insights into the development of next-generation anti-SARS-CoV-2 therapeutics (Fig. 1). These findings highlight the potential of targeting the M protein as a novel strategy to address existing gaps in antiviral treatment.

The coronavirus M protein is the most abundant viral structural protein and serves as a critical driver of viral assembly and morphogenesis. M protein exists in two distinct conformational states, M_{short} and M_{long}, and conformation equilibrium between these states are essential for the assembly process. By interacting with key viral components, including the nucleocapsid protein and genomic RNA, the M protein induces membrane curvature, a critical step in facilitating viral particle formation and maturation[3]. Due to its central role in viral assembly and its high degree of conservation across sarbecoviruses, the M protein emerges as a promising therapeutic target. However, current anti-SARS-CoV-2 therapies predominantly target the RdRp and the M^{pro}, while the development of small-molecule inhibitors targeting M protein remains underexplored. This gap highlights the urgent need for novel therapeutic strategies targeting the M protein, which could offer substantial clinical benefits in the treatment of coronavirus infections.

The identification of potential antiviral compounds targeting SARS-CoV-2 and related coronaviruses has been advanced through phenotypic screening and medicinal chemistry optimization. **JNJ-9676** was identified by Van Loock and Draghia-Akli's team through high throughput-based phenotypic screening of the Janssen proprietary compound library[1], while **CIM-834** was discovered by Neyts and Chaltin's group following an imaging-based high-throughput, high-content phenotypic screening against SARS-CoV-2, coupled with medicinal chemistry optimization[2]. Both compounds were evaluated across multiple cell lines and virus strains to assess antiviral activity and selectivity.

JNJ-9676 demonstrated potent antiviral activity against SARS-CoV-2 and its variants, with EC_{50} values of 14−22 nmol/L against SARS-CoV-2 B1 in A549-hACE2 and VeroE6-eGFP cells. Against the Omicron and Delta variants, it exhibited EC_{50} values of 26 and 14 nmol/L, respectively. **CIM-834** showed average EC_{50} values of 112 and 84 nmol/L in VeroE6-GFP and $A549^{ACE2+TMPRSS2}$ cells, respectively, and effectively inhibited SARS-CoV-2 isolates with activity comparable to nirmatrelvir. Additionally, **CIM-834** significantly reduced viral yield in primary human nasal epithelial cells infected with the SARS-CoV-2 B.1.1.7 variant. The two compounds also exhibited broad-spectrum activity against SARS-CoV, with **JNJ-9676** achieving

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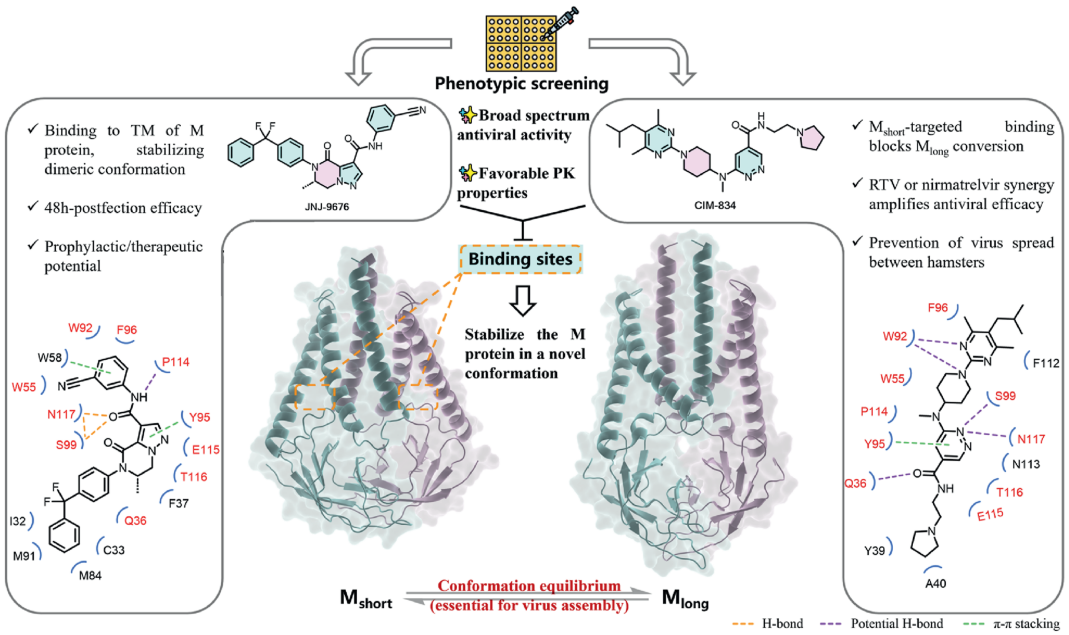

Figure 1 The chemical structures of **JNJ-9676** and **CIM-834**, along with their characteristic comparisons, 2D binding mode diagrams with the M protein, and the relationship between the two forms of the M protein.

EC_{50} values of 16−21 nmol/L and **CIM-834** showing EC_{50} values of 530−640 nmol/L across different cell lines. Furthermore, **JNJ-9676** demonstrated activity against SARS-like animal coronaviruses, including bat WIV-1, bat SHC014, and pangolin coronavirus (Pg-CoV), with EC_{50} values ranging from 4 to 6 nmol/L. Collectively, these findings highlight **JNJ-9676** and **CIM-834** as promising broad-spectrum antiviral agents with potential applications against SARS-CoV-2, its variants, and related coronaviruses.

Next, both studies performed *in vitro* resistance selection (IVRS) assays to determine the target of **JNJ-9676** and **CIM-834**. The results showed that as drug concentration increased, mutations arose in the viral M protein, suggesting that **JNJ-9676** and **CIM-834** target the M protein. This conclusion was further validated by subsequent offline affinity-selection−mass spectrometry (AS−MS). However, the resistance mutation profiles of **JNJ-9676** and **CIM-834** exhibited some differences. The primary drug resistance mutations of **JNJ-9676** were P132S, S99A, and N117K, which increased the EC_{50} value by 43-, 97-, and 145-fold, respectively. For **CIM-834**, the predominant drug resistance mutation was P132S, leading to a 58-fold increase in the EC_{50} value. Additionally, M91K and N117K mutations were observed, but these mutations resulted in only a 2.6- and 5.4-fold increase in the EC_{50} value, respectively, indicating a relatively minor contribution to **CIM-834** drug resistance. These divergent mutation patterns likely reflect differences in the binding sites and mechanisms of action of the two compounds.

To gain deeper insights into the mechanisms of action of **JNJ-9676** and **CIM-834**, as well as their interactions with the M protein, both studies utilized cryo-electron microscopy (cryo-EM) to analyze the ligand-protein complexes. The two compounds functioned by interfering with the conformational dynamics of the M protein, though their specific mechanisms differ. **JNJ-9676** bound to the transmembrane domain (TM) of M protein, stabilizing its dimeric conformation and inhibiting viral assembly. **CIM-834** specifically bound to and stabilizes M_{short}, preventing its transition to M_{long}, thereby blocking the multimerization necessary for viral particle assembly and exerting antiviral effects. The ligand−protein complex structures revealed that the binding sites of **JNJ-9676** and **CIM-834** share significant similarity, with approximately 60% of the amino acid residues in the M protein's binding pocket being conserved (Fig. 1). This similarity likely underlies the overlapping mechanisms of the two compounds. However, some differences in their binding interactions were observed: **JNJ-9676** engaged in two $\pi-\pi$ stacking interactions with W58 and Y95, as well as hydrogen bonds with S99, N117, and a potential hydrogen bond with P114. **CIM-834**, on the other hand, formed a $\pi-\pi$ stacking interaction with Y95 and putative hydrogen bonds with Q36, W92, S99, N117, and E115 (Fig. 1). The differences in binding modes likely accounted for the distinct sensitivities of the compounds to resistance mutations, as observed in the resistance profiling studies. These findings provide structural insights into the mechanisms of these antiviral agents and may guide the design of next-generation inhibitors targeting the M protein.

After investigating the antiviral effect and mechanisms of action, both studies further assessed the *in vivo* efficacy using animal models. Researchers first conducted pharmacokinetic investigations, and the results demonstrated that **JNJ-9676** and **CIM-834** exhibit favorable oral bioavailability and metabolic stability, enabling the evaluation of their antiviral efficacy in animals. **JNJ-9676** showed significant antiviral effects in the Syrian golden hamster model, significantly reducing viral load and infectious virus titers in the lungs under both prophylactic and therapeutic dosing regimens with a lowest efficacious concentration of 25 mg/kg BID, while also alleviating histopathological damage. Notably, **JNJ-9676** retained its efficacy even when administered 48 h post-infection. For **CIM-834**, significant reductions in infectious viral titers (by > 3 $\log_{10}$ and >4 $\log_{10}$, respectively) and viral RNA levels (by 2.5 $\log_{10}$ and 3.4 $\log_{10}$,

respectively) were observed, whether administered as monotherapy or in combination with ritonavir (RTV) or nirmatrelvir. Combination therapy further improved lung histopathology in both **CIM-834**/RTV and **CIM-834**/nirmatrelvir groups. Additionally, the **CIM-834**/RTV group demonstrated the ability to prevent viral transmission among hamsters. These findings collectively highlight **JNJ-9676** and **CIM-834** as promising clinical candidates for the treatment of SARS-CoV-2 infections, with potential applications in both monotherapy and combination regimens.

In conclusion, **JNJ-9676** and **CIM-834**, as the coronavirus M protein assembly inhibitors, have exhibited robust antiviral efficacy in both *in vitro* and *in vivo* studies. These findings not only highlight the potential of the M protein as a promising target for antiviral drug development but also elucidate novel mechanisms of action and strategic directions for combating coronavirus infections. Moreover, the integration of phenotypic screening with multiple target validation techniques provides a replicable and systematic approach for the discovery and optimization of other antiviral drugs, thereby accelerating progress in the field of antiviral research.

However, the ongoing emergence of drug resistance continues to pose a significant challenge in the development of effective antiviral drugs. Resistance mutations have been identified for both **JNJ-9676** and **CIM-834**, with **JNJ-9676** exhibiting greater susceptibility to these mutations. For instance, the N117K mutant strain reduces the antiviral activity of **JNJ-9676** by more than 100-fold, whereas the impact on **CIM-834** is relatively modest (5.4-fold). Structural analysis of the complexes formed by **JNJ-9676** and **CIM-834** with the M protein offers valuable insights that facilitate addressing the issue of their drug resistance. Specifically, based on the target adaptability of M protein and structural biology information, the covalent binding strategy can be utilized to target key nucleophilic amino acid residues, such as Y95, S99, and C33, within the binding pocket. Through the rational incorporation of covalent warheads, covalent inhibitors are systematically designed to identify highly efficient M protein assembly inhibitors with potential efficacy against drug resistance[4]. Additionally, targeting new ligand-binding sites of important proteins is considered to be an effective strategy to improve affinity and drug−resistance profiles, which has been fully reflected in the study of SARS-CoV-2 papain-like protease inhibitor, which will also provide inspiration for the development of M protein inhibitors[5,6]. Finally, "event-driven" mechanism-based targeted protein degradation technology demonstrates significant potential in combating drug resistance[7,8]. Given that the M protein plays multifunctional roles in the coronavirus replication process, its degradation could potentially eliminate all associated functions. Exploring the synergistic use of M protein assembly inhibitors alongside other antiviral drugs targeting distinct mechanisms represents another effective strategy to minimize the risk of drug resistance. Notably, as the first-in-class molecules specifically targeting the M protein, **JNJ-9676** and **CIM-834** exhibit considerable promise for further development. Leveraging artificial intelligence (AI)-driven intelligent drug design to optimize the pharmacokinetic properties of these compounds could expedite their transition into clinical candidate drugs[9]. Overall, these two groundbreaking studies not only provide novel solutions for addressing the ongoing COVID-19 pandemic but also offer promising strategies for mitigating potential cross-species transmission of coronaviruses in the future.

Additionally, the specific mechanisms by which viruses develop resistance to these novel inhibitors warrant further investigation. So far, only the correlation between resistance mutation sites (such as P132S, N117K, and S99A) and reduced drug sensitivity has been mentioned, without a deep elucidation of the molecular basis for resistance phenotype formation. For instance, it is unclear how the P132S mutation precisely affects the conformational changes and oligomerization of M protein, thereby altering the drug binding pocket, and whether resistance mutations impact the interaction between viral membrane proteins and other accessory proteins.

Future research should prioritize elucidating resistance mechanisms, which will help clarify the dynamic functions of M protein in the viral life cycle and provide crucial insights for refining drug design[10,11]. Systematic functional analyses of resistance mutants, complemented by high-resolution cryo-EM structural studies and molecular dynamics simulations, can illuminate how mutations alter drug−target binding affinity and protein conformational stability. Meanwhile, evaluating the effects of resistance mutations on viral transmissibility and pathogenicity, as well as infection dynamics in animal models, will enable an assessment of the potential risks posed by resistant viruses. These studies will lay the foundation for developing next-generation M protein-targeted drugs with higher resistance barriers and provide scientific evidence for devising clinical treatment strategies, ensuring effective containment of resistance emergence and spread when combating SARS-CoV-2 and its variants.

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Author contributions

Dazhou Shi: Writing - original draft, review & editing. Chunhua Ma: Funding acquisition, Validation. Shujing Xu: Writing - review & editing, Conceptualization. Peng Zhan: Writing-review & editing, supervision, funding acquisition, conceptualization.

Conflicts of interest

The authors declare no conflicts of interest.

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