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Current Opinion in Virology

Antiviral drugs specific for coronaviruses in preclinical development

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Coronaviruses are positive stranded RNA viruses that cause respiratory, enteric and central nervous system diseases in many species, including humans. Until recently, the relatively low burden of disease in humans caused by few of these viruses impeded the development of coronavirus specific therapeutics. However, the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV), and more recently, Middle East respiratory syndrome coronavirus (MERS-CoV), has impelled the development of such drugs. This review focuses on some newly identified SARS-CoV inhibitors, with known mechanisms of action and their potential to inhibit the novel MERS-CoV. The clinical development of optimized versions of such compounds could be beneficial for the treatment and control of SARS-CoV, the current MERS-CoV and other future SARS-like epidemics.

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Current Opinion in Virology 2014, **8**:45–53

This review comes from a themed issue on **Antivirals and resistance**

Edited by **Luis Menéndez-Arias** and **Douglas D Richman**

For a complete overview see the Issue and the Editorial

Available online 2nd July 2014

http://dx.doi.org/10.1016/j.coviro.2014.06.002

Introduction

In September 2012, a novel coronavirus (CoV) called Middle East respiratory syndrome CoV (MERS-CoV), was isolated as the causative agent of a severe pneumonia in several patients in the Middle East [1]. Globally, as of May 16, 2014, WHO has been informed of a total of 614 laboratory-confirmed cases of infection with MERS-CoV (including 181 deaths) primarily in the Middle East (Saudi Arabia, Jordan, Qatar, Oman, Kuwait, and the United Arab Emirates), but also in Europe (the UK, France, Italy, Germany, and Greece), North Africa (Tunisia and Egypt), Asia (Malaysia) and the United States of America (http://www.who.int/csr/don/2014_05_16_mers/en/, http://www.cdc.gov/coronavirus/mers/). This CoV is closely related to severe acute respiratory syndrome CoV (SARS-CoV), an epidemic that was short-lived but alarming in 2002–2003 that resulted in approximately 8000 cases and 800 deaths.

SARS-CoV and MERS-CoV both belong to the family Coronaviridae, which are enveloped, positive-stranded RNA viruses with approximately 30,000 nucleotides [2••]. CoVs represent the largest RNA viruses. For the well-characterized SARS-CoV, two overlapping open reading frames (ORF1a and ORF1b), encompass approximately two-thirds of the genome. A translational read-through by a −1 ribosomal frameshift mechanism allows the translation of the overlapping reading frames into a single polyprotein pp1ab, whereas, translation without the −1 ribosomal frameshift mechanism produces pp1a. The polyproteins are later cleaved by two viral proteinases, 3C-like protease (3CLP) and papain-like protease (PLP), to yield non-structural proteins essential for viral replication [3,4]. The remaining one-third of the genome encodes structural proteins of the virus, which include the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins [5,6].

On the basis of phylogenic analyses, evolutionary studies have shown that SARS-CoV originated most likely from bats. It has been reported to be transmitted to humans by aerosols through intermediate hosts like palm civets infected by the virus [7–9]. Therefore, the zoonosis of CoV is a threat, due to its ability of interspecies transfer into human population. This has been recapitulated with the novel MERS-CoV, as recent studies have suggested that bats and dromedary camels serve as a reservoir for this virus [10–15]. MERS-CoV shows SARS-like symptoms following human infections, which include malaise, rigors, fatigues and high fevers, indications similar to influenza, but later progresses to atypical pneumonia in most cases [16].

Although, many antiviral agents have been identified to inhibit SARS *in vitro*, there are presently, no approved antiviral agents or vaccines available to tackle any potential SARS or SARS-like outbreaks, such as MERS. Different parts of the virus, that are deemed viable targets include, 3CLP, PLP, RNA-dependent RNA polymerase (RdRp) and the 5′–3′ helicase [17,18•,19]. Other possible

targets include E protein (Orf4), M protein (Orf6), and N protein (Orf9) [17]. This review focuses on the published antiviral inhibitors of coronaviruses, with SARS-CoV being considered as the primary virus. The inhibitors include replication and entry inhibitors, which can be developed for therapeutic purposes, not just against SARS, but other coronaviruses, including the novel MERS-CoV.

Replication inhibitors

Protease inhibitors

The first coronavirus proteins that have been studied in detail include viral proteinases, namely the papain-like protease (PLP) or nsp3 and the 3C-like protease (3CLP, nsp5 or Mpro), which cleave the polyprotein into individual polypeptides that are required for replication and transcription [3,4]. Following the translation of the messenger RNA to yield the polyproteins, the 3CLP is first auto-cleaved from the polyproteins to become a mature enzyme. The 3CLP further cleaves all the 11 remaining downstream non-structural proteins. Hence, 3CLP is an essential viral protein for the viral replication cycle, and as a result becomes an attractive target for anti-SARS drug development [20,21••,22]. 3CLP inhibitors are among the first SARS-CoV inhibitors that were discovered by screening compound libraries using an assay that utilizes a fluorogenic peptide as the substrate and with structure-based design on the basis of the crystal structures of the product-bound form of 3CLP [23–25]. The compounds identified include zinc or mercury conjugates [26,27], C2-symmetric diols [28,29], peptidomimetic α,β-unsaturated esters [30], anilides [31], benzotriazole [32], N-phenyl-2-(2-pyrimidinylthio)acetamide [33], biphenyl sulfone [34], glutamic acid and glutamine peptides possessing a trifluoromethylketone group [35], pyrimidinone [36], and pyrazole analogs that can also inhibit 3Cpro of picornaviruses CV-B3 (coxsackievirus), EV-71 (enterovirus) and RV-14 (rhinovirus) (coronavirus and picornavirus dual inhibitors) [24,25]. The names and chemical structures of some of the published 3C-protease inhibitors are shown in Figure 1.

The papain like protease (PLP) is also an essential component of the SARS-CoV replication machinery. PLP is the nsp3 protein which is part of the synthesized ORF1a polyprotein during replication. nsp3 cleaves protease recognition sites between nsp1/2, nsp2/3 and nsp3/4 [37]. In addition to its protease activity, nsp3 has been shown to have deubiquitination, and interferon antagonist activities *in vitro* [38]. Since its homologues are found in all coronaviruses, it has also been proposed to be a good target for drug discovery for both SARS-CoV and other human coronaviruses.

Recently, Frieman *et al.* [39] developed a yeast-based assay to screen for small molecules that block SARS-CoV replication on the basis of their inhibition of nsp3 or PLP. The basis for the screen was that stimulated expression of nsp3 in *Saccharomyces cerevisiae* causes a pronounced slow growth phenotype. Using this principle, they screened a small molecule library for compounds that specifically prevented the nsp3-induced slow growth phenotype. These compounds were then validated in cell culture models for efficacy against SARS-CoV replication, as well as the known enzymatic functions of nsp3. The authors found five compounds that reversed the slow growth phenotype in yeast. One of the compounds, NSC158362 (Figure 1g), considerably blocked SARS-CoV replication *in vitro* with an $EC_{50} < 1$ μM. This effect was specific for SARS-CoV replication because no effect on influenza virus replication was observed with up to 50 μM of the inhibitory compound. Another compound, NSC158011, was shown to inhibit nsp3-dependent protease activity in a cell culture assay, but could not prevent virus replication. NSC158362, could not inhibit the protease, deubiquitinase or anti-IFN activities of nsp3, therefore suggesting that the compound may be inhibiting a yet unknown novel activity of nsp3 required for viral replication or may be inhibiting some cellular factors that regulate nsp3 function in infected cells.

Helicase inhibitors

Helicases are proteins that catalyze the separation of duplex oligonucleotides into single strands in an ATP-dependent reaction. On the basis of this activity, helicases can be divided into two types: those that unwind duplexes in a 3′→5′ direction, and those that unwind in a 5′→3′ direction. Helicases require a molecular mechanism for transducing the chemical energy generated by the ATPase activity into an oligonucleotide strand separation and displacement activity [40]. The functions of helicase in positive-sense RNA viruses include nucleic acid separations [41••], the melting of highly stable secondary structures within the genomic RNA in order to increase translational efficiency of the polyprotein [42]. Considering all these helicase functions, viral helicases stand as a strong antiviral target.

The helicase of SARS-CoV is called nsp13. A few potential inhibitors of nsp13 have been identified [19,43–46]. Some of these inhibitors inhibit nsp13 by interfering with its unwinding and ATPase activities. They include the bananins and the 5-hydroxychromone derivatives [43,45]. The bananins are a class of antiviral compounds with an exclusive structural signature that incorporates a trioxa-adamantane moiety covalently bound to a pyridoxal derivative [45]. Six members of this class of compounds were synthesized by Tanner *et al.* [45]. The compounds are bananin, iodobananin, vanillinbananin, ansabananin, eubananin, and adeninobananin. Of the six compounds, bananin, iodobananin, vanillinbananin, and eubananin effectively inhibited the helicase activity of nsp13 by inhibiting the ATPase activity of the helicase with IC_{50} values in the range 0.5–3 μM. Bananin was also