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Protease inhibitors targeting coronavirus and filovirus entry

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ABSTRACT

In order to gain entry into cells, diverse viruses, including Ebola virus, SARS-coronavirus and the emerging MERS-coronavirus, depend on activation of their envelope glycoproteins by host cell proteases. The respective enzymes are thus excellent targets for antiviral intervention. In cell culture, activation of Ebola virus, as well as SARS- and MERS-coronavirus can be accomplished by the endosomal cysteine proteases, cathepsin L (CTSL) and cathepsin B (CTSB). In addition, SARS- and MERS-coronavirus can use serine proteases localized at the cell surface, for their activation. However, it is currently unclear which protease(s) facilitate viral spread in the infected host. We report here that the cysteine protease inhibitor K11777, ((2S)-N-[(1E,3S)-1-(benzenesulfonyl)-5-phenylpent-1-en-3-yl]-2-{[(E)-4-methylpiperazine-1-carbonyl]amino}-3-phenylpropanamide) and closely-related vinylsulfones act as broad-spectrum antivirals by targeting cathepsin-mediated cell entry. K11777 is already in advanced stages of development for a number of parasitic diseases, such as Chagas disease, and has proven to be safe and effective in a range of animal models. K11777 inhibition of SARS-CoV and Ebola virus entry was observed in the sub-nanomolar range. In order to assess whether cysteine or serine proteases promote viral spread in the host, we compared the antiviral activity of an optimized K11777-derivative with that of camostat, an inhibitor of TMPRSS2 and related serine proteases. Employing a pathogenic animal model of SARS-CoV infection, we demonstrated that viral spread and pathogenesis of SARS-CoV is driven by serine rather than cysteine proteases and can be effectively prevented by camostat. Camostat has been clinically used to treat chronic pancreatitis, and thus represents an exciting potential therapeutic for respiratory coronavirus infections. Our results indicate that camostat, or similar serine protease inhibitors, might be an effective option for treatment of SARS and potentially MERS, while vinyl sulfone-based inhibitors are excellent lead candidates for Ebola virus therapeutics.

1. Introduction

Emerging viral diseases pose a unique risk to public health. Ebola virus, severe acute respiratory syndrome coronavirus (SARS-CoV) and members of the Henipavirus genus of paramyxoviruses are all highly pathogenic viruses that have arisen in the past 40 years and caused, or threaten to cause, major outbreaks. New viral threats continue to emerge, most recently demonstrated by a novel beta-coronavirus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which was identified in 2012 (Zaki et al., 2012; Memish et al., 2013; de Groot et al., 2013). There are currently no approved vaccines or therapeutics for many of the highly pathogenic viruses potentially dependent on cathepsins, including Ebola virus, Nipah virus (NiV), MERS-CoV and SARS-CoV. Broad-spectrum antiviral drugs, with overlapping therapeutic indications, would facilitate rapid responses to new or changing pandemic threats, potentially even without precise identification of the agent. Targeting host factors involved in viral entry provides an excellent avenue for such drug development, due to the limited number of pathways involved (Zhou et al., 2011).

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The glycoproteins of corona-, filo- and paramyxoviruses facilitate viral entry into target cells by binding to receptors and by driving fusion of viral and host cell membranes. However, the glycoproteins are synthesized as inactive precursors and depend on activation by host cell proteases to acquire a fusion active form. As a consequence, the respective enzymes are potential targets for broad-spectrum antiviral intervention. Cell culture studies demonstrated that endosomal cysteine proteases, in particular cathepsin B (CTSB) and/or L (CTSL), can activate the glycoproteins of filoviruses, SARS-CoV, other coronaviruses, and NiV and Hendra (HeV) viruses to facilitate entry into certain cell lines. In addition, activation of coronaviruses can also be accomplished by TMPRSS2, or other serine proteases located at the cell surface, or secreted into the extracellular space (Simmons et al., 2013). However, the respective roles of endosomal and cell surface proteases in viral spread in the infected host is unknown.

The development of protease inhibitors able to inhibit CTSL, CTSB and related proteases would be an excellent starting point for development of broad-spectrum antiviral therapies (Zhou et al., 2011). We describe here the discovery of K11777 and its related compounds, as broad-spectrum antivirals targeting endosomal proteases involved in viral entry. K11777, a cysteine protease inhibitor, blocked infection when viral entry did not require activating serine proteases, as is the case with ebolavirus (EBOV). K11777 also fully inhibited coronavirus infection, but only when target cell lines lacking activating serine proteases were used. If cells expressed cell-surface serine proteases known to activate coronaviruses, both K11777 and a serine protease inhibitor, such as camostat were required for full inhibition. Thus, both compounds were deployed to examine which activation pathway is predominant *in vivo*. Camostat displayed antiviral activity in a pathogenic animal model for SARS-CoV infection, indicating that serine protease inhibitors are suitable for treatment of SARS and potentially MERS. The predicted effect of K11777 and related cysteine protease inhibitors versus Ebola virus *in vivo* must await studies in approved biocontainment facilities.

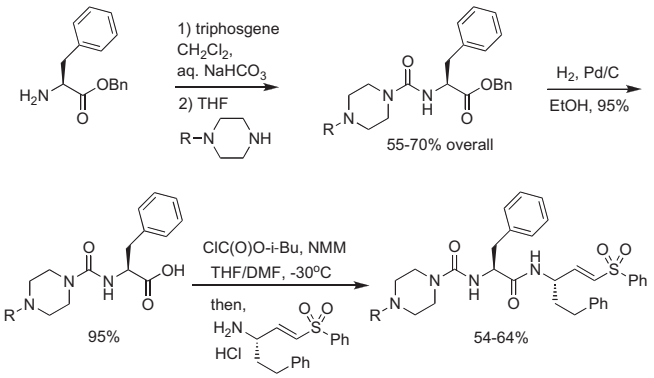
2. Materials and methods

2.1. Libraries and commercial compounds

The cysteine protease inhibitor library screened in this work has been described elsewhere (Ang et al., 2011). Briefly, the library includes ~2100 electrophilic cysteine protease inhibitors of various chemotype (glycine nitriles, ketobenzoxazoles, ketooxadiazoles, vinylsulfones, etc.), which were synthesized during the course of industrial drug discovery programs targeting human cathepsins (Palmer et al., 1995, 2005, 2006; Rydzewski et al., 2002). Camostat mesylate, leupeptin, bafilomycin A1, ammonium chloride, and chloroquine were purchased from Sigma–Aldrich.

2.2. Synthesis of vinylsulfone cysteine protease inhibitors

K11777 and novel P3 derivatives were synthesized according to the general approach described previously (Jaishankar et al., 2008) and as illustrated here (Scheme 1). The *N*-substituted piperazines were obtained from commercial sources or (for R = cyclopentyl and cyclopropylmethyl) were prepared by reductive amination of Boc-protected piperazine followed by treatment with HCl in dioxane (51–53% over two steps). We find that the final coupling of P3/P2 carboxylic acid to vinylsulfone amine is best accomplished via the mixed anhydride, as this minimized epimerization of the phenylalanine side chain. Final vinylsulfone analogs were >95% pure as judged by LC/MS analysis. Characterization data for final analogs is provided below.



Scheme 1. Synthesis of K11777 and P3-modified vinylsulfone analogs.

2.2.1. (2S)-N-[(1E,3S)-1-(benzenesulfonyl)-5-phenylpent-1-en-3-yl]-2-{[(E)-4-ethylpiperazine-1-carbonyl]amino}-3-phenylpropanamide (SMDC-256122)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93–7.91 (m, 2H), 7.78–7.68 (m, 1H), 7.68–7.58 (m, 2H), 7.37–7.25 (m, 8H), 7.16–7.14 (m, 2H), 6.90 (dd, J = 4.8, 15.2 Hz, 1H), 6.35–6.31 (m, 2H), 4.67 (br. S., 1H), 4.52 (d, J = 6.8 Hz, 1H), 4.13 (br. S., 3H), 3.50 (br. S., 2H), 3.39 (br. S., 2H), 3.21–3.05 (m, 4H), 2.74–2.549 (m, 4H), 1.97–1.90 (m, 2H), 1.37 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 156.6, 145.2, 140.1, 139.89, 136.4, 133.14, 130.14, 129.0, 128.9, 128.49, 128.29, 128.09, 127.3, 126.8, 125.9, 76.7, 76.4, 55.7, 54.2, 48.8, 47.8, 43.8, 37.8, 35.4, 31.4, 18.0; MS m/z = 589 $[M+H]^+$.

2.2.2. (2S)-N-[(1E,3S)-1-(benzenesulfonyl)-5-phenylpent-1-en-3-yl]-2-{[(E)-4-(propan-2-yl)piperazine-1-carbonyl]amino}-3-phenylpropanamide (SMDC-256123)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93–7.90 (m, 2H), 7.72–7.68 (m, 1H), 7.63–7.59 (m, 2H), 7.34–7.20 (m, 8H), 7.14–7.12 (m, 2H), 6.85 (dd, J = 4.8, 15.2 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 6.18 (dd, J = 1.7, 15.1 Hz, 1H), 5.05 (d, J = 7.3 Hz, 1H), 4.68 (m, 1H), 4.56 (d, J = 7.3 Hz, 1H), 3.45–3.28 (m, 4H), 3.10 (dd, J = 2.6, 7.3 Hz, 2H), 2.78–2.72 (m, 1H), 2.66–2.59 (m, 2H), 2.58–2.44 (m, 4H), 1.97–1.91 (m, 1H) 1.85–1.81 (m, 1H) 1.08 (m, 6H); MS m/z = 603 $[M+H]^+$.

2.2.3. (2S)-N-[(1E,3S)-1-(benzenesulfonyl)-5-phenylpent-1-en-3-yl]-2-{[(E)-4-propylylpiperazine-1-carbonyl]amino}-3-phenylpropanamide (SMDC-256157)

^{1}H NMR (400 MHz $CDCl_3$) δ = 7.85–7.83 (m, 2H), 7.67–7.61 (m, 1H), 7.55 (t, J = 7.6 Hz, 2H), 7.25–7.16 (m, 8H), 7.08 (d, J = 7.0 Hz, 2H), 6.79 (d, J = 4.8 Hz, 1H), 6.83 (d, J = 4.8 Hz, 1H), 6.16 (dd, J = 1.6, 15.0 Hz, 1H), 5.56 (s, 1H), 5.19 (br. S., 1H), 4.62–4.60 (m, 1H), 4.47 (d, J = 7.5 Hz, 1H), 3.57–3.51 (m, 2H), 3.37–3.27 (m, 4H), 3.07 (d, J = 7.5 Hz, 2H), 2.65–2.48 (m, 2H), 2.37–2.22 (m, 6H), 1.93–1.72 (m, 3H), 1.52–1.39 (m, 4H), 0.93–0.85 (m, 3H); MS m/z = 603 $[M+H]^+$.

2.2.4. (2S)-N-[(1E,3S)-1-(benzenesulfonyl)-5-phenylpent-1-en-3-yl]-2-{[(E)-4-phenylpiperazine-1-carbonyl]amino}-3-phenylpropanamide (SMDC-256158)

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.94–7.82 (m, 2H), 7.72–7.60 (m, 3H), 7.37–7.21 (m, 10H), 7.12 (d, J = 7.5 Hz, 2H), 7.00–6.96 (m, 3H), 6.87 (dd, J = 15.0 Hz, 4.4, 1H), 6.79 (d, J = 8.2 Hz, 1H), 6.19 (d, J = 15.0 Hz, 1H), 5.23 (d, J = 7.1 Hz, 1H), 4.69–4.59 (m, 2H), 3.57–3.49 (m, 4H), 3.17–311 (m, 6H), 2.66–2.57 (m, 2H), 295–1.78 (m, 2H); MS m/z = 637 $[M+H]^+$.