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Quinoxaline-based inhibitors of Ebola and Marburg VP40 egress

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ABSTRACT

We prepared a series of quinoxalin-2-mercapto-acetyl-urea analogs and evaluated them for their ability to inhibit viral egress in our Marburg and Ebola VP40 VLP budding assays in HEK293T cells. We also evaluated selected compounds in our bimolecular complementation assay (BiMC) to detect and visualize a Marburg mVP40–Nedd4 interaction in live mammalian cells. Antiviral activity was assessed for selected compounds using a live recombinant vesicular stomatitis virus (VSV) (M40 virus) that expresses the EBOV VP40 PPxY L-domain. Finally selected compounds were evaluated in several ADME assays to have an early assessment of their drug properties. Our compounds had low nM potency in these assays (e.g., compounds **21**, **24**, **26**, **39**), and had good human liver microsome stability, as well as little or no inhibition of P450 3A4.

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The 2014–2015 outbreak of Ebola in western Africa resulted in over 28,000 infected individuals and over 11,000 deaths (WHO: Ebola situation report 2015). This unprecedented epidemic has spurred a call to action on new, cost effective therapies that combat this deadly pathogen. Among the efforts are several vaccines and antiviral candidates.[1–3] However the current vaccines in clinical trials are not a complete defense. They must be given pre-exposure and would not be effective against other RNA viruses such as Marburg and Lassa[4] that also cause lethal hemorrhagic fever symptoms. Furthermore, oral antiviral agents used alone or in combination may be of value for individuals who respond adversely to the vaccine, and could be of value as prophylactic agents for individuals deemed to be in high risk situations such as military or healthcare workers. Therefore, effective therapeutics are needed to safeguard the largely immunologically naive human population by providing immediate protection.

We have discovered two novel series of small molecule early leads that inhibit RNA virus budding.[5] Our approach does not rely solely on viral targets, but instead focuses on a critical virus-host interaction required by PPxY motif-containing viruses for efficient egress and spread. We hypothesize that targeting a virus–host interaction necessary for efficient virus egress and dissemination will greatly diminish or eliminate the occurrence of drug resistant viral mutations. Importantly, as these virus-host interactions represent a common mechanism in a range of RNA viruses, we predict that they represent an Achilles' heel in the life cycle of RNA virus pathogens.

Late budding domains (containing PPxY and PTAP motifs) are highly conserved in the matrix proteins of a wide array of RNA viruses (e.g., filoviruses, arenaviruses, rhabdoviruses, paramyxoviruses, henipaviruses, and retroviruses) and represent broad-spectrum targets for the development of novel antiviral therapeutics.[6–16] For example, the filovirus VP40, arenavirus Z, and rhabdovirus M proteins play central and sufficient roles in virion assembly and egress, due in part to the presence of a PPxY L-domain.[16–23] Efficient egress of VLPs depends on viral L-domain mediated recruitment of host proteins required for complete virus-cell separation or pinching-off of virus particles.[7–9,11–13,15,16,24] In this regard, the viral matrix protein VP40 (for filoviruses Ebola and Marburg) or Z (for arenavirus Lassa) contains a PPxY L-domain motif that recruits the mammalian cellular protein Nedd4, which is a WW-domain containing cellular E3 ubiquitin ligase associated with the host ESCRT1 complex (endosomal sorting complex required for transport), and this interaction is critical for efficient budding of filoviruses, arenaviruses, and rhabdoviruses.[9,11,15,16,18,22,24–34]

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Herein we describe our current efforts to exploit the viral PPxY–host WW domain interaction to obtain broad-spectrum RNA antiviral therapeutics. This paper focuses on SAR around our lead **1** (Table 1) which we described in detail previously.[5] We used our Marburg VP40 VLP budding assay in HEK293T cells as our primary assay for the SAR analogs compiled in Tables 1–3. This assay measures the % inhibition of viral VP40 VLP egress from the cell versus DMSO control. In addition we provide Western analysis for selected compounds using this assay and the Ebola VP40 VLP budding assay (Figs. 2 and 3). We also evaluated selected compounds in our bimolecular complementation assay (BiMC)[35] to detect and visualize a Marburg VP40–Nedd4 interaction in live mammalian cells in the absence or presence of the indicated inhibitors in Figure 1. We then assessed antiviral activity for selected compounds using a live recombinant vesicular stomatitis virus (VSV-M40 virus) that expresses the EBOV VP40 PPxY L-domain and flanking residues[36] in Figure 4. Relative cell viability was validated by the MTT assay at concentration ranges of 0.01–1.0 μM on VeroE6 and HEK293T cells (data presented in Supplementary material section). Finally selected compounds were evaluated in several ADME assays (Table 4) to have an early assessment of their drug properties.

Compounds **2**–**13** (Table 1) and **17**–**52** (Tables 2 and 3) were prepared according to Schemes 1 and 2. While compounds **1** and **14**–**16** were originally purchased from Ambinter (Orléans, France), larger quantities of **1** were synthesized by us via the methods outlined in Scheme 1. Experimental and analytical information for compounds **1**–**13** and **17**–**52** are described in the Supplementary material section.

Referring to Scheme 1, target compounds in Tables 1–3 were generally prepared by alkylation of quinoxaline thiols **55** with α-chloro-acetamidoureas **57**. The alkylating agents (**57**) were in turn obtained via reaction of commercially available anilines or heteroaromatic amines **56** with commercially available chloroacetyl isocyanate. In most cases, the quinoxaline thiol **55** was commercially available but in a few examples ($R^1 = CF_3$ and Et) we prepared this thiol by converting congeners **54** ($R^1 = CF_3$ and Et) using P_2S_5 and pyridine.[37] Compound **54** where $R^1 = CF_3$ was prepared by reaction of *o*-phenylenediamine with ethyl trifluoropyruvate[38] and **54** where R^1 = Et is commercially available.

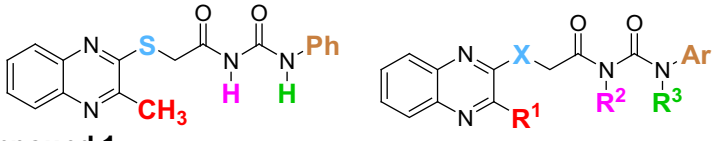
Several of the target compounds in Table 1 were prepared by alternative routes highlighted in Scheme 2. For compounds with X = O (e.g., **9** and **10**), we started with commercially available 2-chloro-3-methyl-quinoxaline (**58**) since reaction of compounds **54** with **57** led to N-alkylation products. Substitution of the chlorine of **58** with methyl glycolate, and subsequent saponification of the methyl ester led to acid **59**. Conversion to the primary amide **60** and subsequent reaction with the requisite aryl isocyanate[39,40] led to target compounds **9** and **10**. Preparation of *N*-methylated target compound **12** ($R^2 = CH_3$) was accomplished by alkylation of **20** with methyl iodide. Thioether **20** was also used to prepare sulfone analog **8** via mCPBA oxidation conditions.

We examined the X and R^1–R^3 substituent changes of **1** in Table 1. With the exception of analogs **9** and **10**, the compounds **2**–**8**, **11**–**13** in this table had little or no inhibition of Marburg VLP egress at 1 μM or greater. SAR highlights are summarized below with the full data set listed in the Supplementary section (Table 1S). Tables 2 and 3 focused on variation of the terminal aryl substituent of **1**.

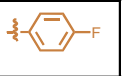
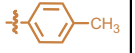
Referring to Table 1, we have not found a suitable replacement of methyl for the **R^1** substituent on the quinoxaline moiety of **1**. All replacements either smaller, H (**2**) or larger, CF_3 (**3**, **4**), Et (**5**, **6**) or CH_2Ph (**7**) were less active or not active at the 1 μM concentration in the Marburg VLP inhibition assay. The CF_3 and Et groups did provide compounds with greater stability to mouse liver microsomes relative to the methyl congeners however (vide infra).

We replaced the sulfur atom of compound **1** (**X** = S) with a SO_2 moiety (**8**) or an O atom (**9**, **10**). While compound **8** did not show activity at 1 μM, the ether analogs **9** and **10** were approximately a

Table 1
Analogs of **1**. Examination of changes in highlighted areas



Compound 1

No.	R^1	X	R^2	R^3	Ar	No.	R^1	X	R^2	R^3	Ar
2	H	S	H	H		8	CH_3	SO_2	H	H	
3	CF_3	S	H	H		9	CH_3	O	H	H	
4	CF_3	S	H	H		10	CH_3	O	H	H	
5	Et	S	H	H		11	CH_3	S	H	CH_3	
6	Et	S	H	H		12	CH_3	S	CH_3	H	
7	CH_2Ph	S	H	H		13	CH_3	S	H	NR^3Ar =	

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