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Synthesis of 1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole and its *in vitro* and *in vivo* efficacy against *Hantavirus*

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ABSTRACT

There are no FDA approved drugs for the treatment of hemorrhagic fever with renal syndrome (HFRS), a serious human illnesses caused by hantaviruses. Clinical studies using ribavirin (RBV) to treat HFRS patients suggest that it provides an improved prognosis when given early in the course of disease. Given the unique antiviral activity of RBV and the lack of other lead scaffolds, we prepared a diverse series of 3-substituted 1,2,4-triazole-β-ribosides and identified one with antiviral activity, 1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR). ETAR showed an EC_{50} value of 10 and 4.4 μM for Hantaan virus (HTNV) and Andes virus, respectively. ETAR had weak activity against Crimean Congo hemorrhagic fever virus, but had no activity against Rift Valley fever virus. Intraperitoneally delivered ETAR offered protection to suckling mice challenged with HTNV with a ~25% survival at 12.5 and 25 mg/kg ETAR, and a MTD of 17.1 ± 0.7 days. ETAR was phosphorylated in Vero E6 cells to its 5′-triphosphate and reduced cellular GTP levels. In contrast to RBV, ETAR did not increase mutation frequency of the HTNV genome, which suggests it has a different mechanism of action than RBV. ETAR is an exciting and promising lead compound that will be elaborated in further synthetic investigations as a framework for the rational design of new antivirals for treatment of HFRS.

1. Introduction

Despite efforts to develop vaccines and antiviral drugs, effective therapeutics for treatment of most hemorrhagic fever viruses remain largely unavailable (Andrei and De Clercq, 1993; Bangash and Khan, 2003; Bronze and Greenfield, 2003; De Clercq, 2005; Maes et al., 2004). Hantaviruses are globally distributed and several members of the genus cause deadly human illnesses such as hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS) (Schmaljohn and Hjelle, 1997). Old World hantaviruses, Hantaan virus (HTNV) and Puumala virus, are responsible for most HFRS cases in Asia and Europe, whereas the New World hantaviruses, Sin Nombre virus (SNV) and Andes virus (ANDV), are responsible for the majority of HPS cases in North and South America, respectively (Peters et al., 1999). In striking contrast to all other HPS and HFRS-causing viruses (Vitek et al., 1996; Wells et al., 1997), ANDV represents the first hantavirus associated with person-to-person transmission in Argentina and Chile (Chaparro et al., 1998; Enria et al., 1996; Lopez et al., 1996; Martinez et al., 2005; Padula et al., 1998). While ribavirin (RBV; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) has shown efficacy in treating HFRS patients in China (Huggins et al., 1991), its potential efficacy is still unknown for HPS cases (Chapman et al., 1999; Mertz et al., 2004).

In addition to *Hantavirus*, several other genera in the family *Bunyaviridae* cause hemorrhagic fever disease in humans. Crimean Congo hemorrhagic fever virus (CCHFV) and Rift Valley fever virus (RVFF) reside in the *Nairovirus* and *Phlebovirus*, respectively, and have mortality rates from 1% (RVFV) to 5-40% (CCHFV). Hantaviruses are enzootic viruses of wild rodents and cause persistent infections without apparent disease symptoms in their natural hosts (Botten et al., 2000; Botten et al., 2002; Compton et al., 2004; Lee et al., 1981; Yanagihara et al., 1985). However, the basic genome structure and replication cycles of members of the family *Bunyaviridae* share many similarities (Schmaljohn and Hooper, 2001), and therefore, antiviral drugs may prove effective for more

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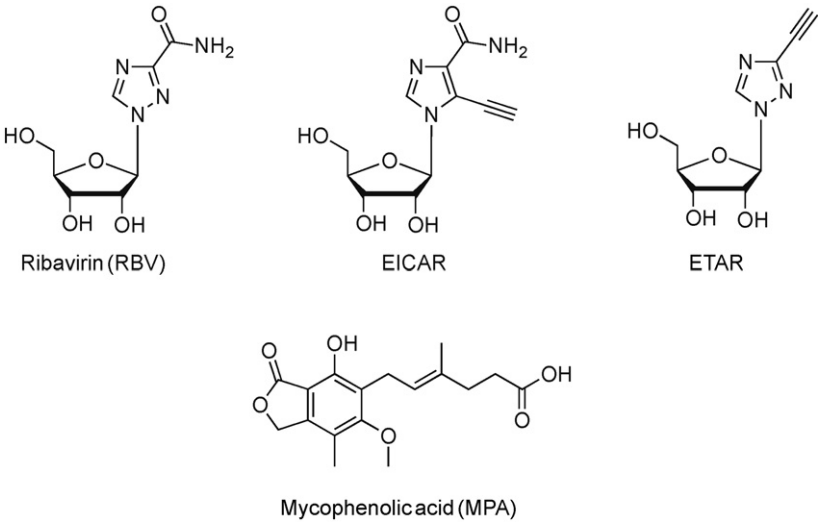
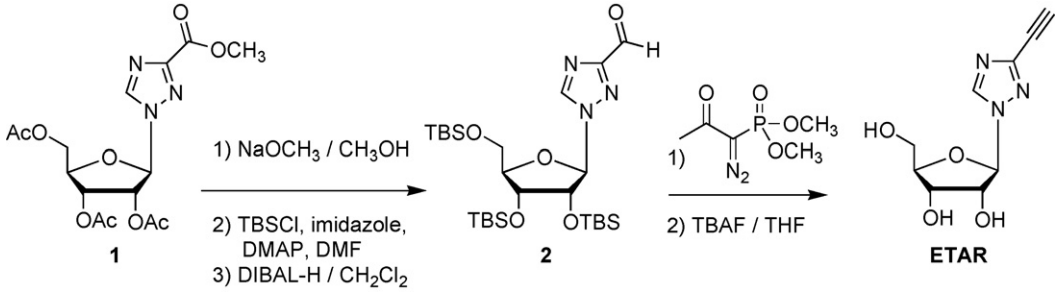


Fig. 1. Structures of RBV, MPA, EICAR, and ETAR.



Scheme 1. Synthesis of ETAR.

than one genus. All the *Bunyaviridae* have three negative-sense, single-stranded RNA segments (S, M, and L), which encode the nucleocapsid (N), two glycoproteins (G_N and G_C) and the L protein, respectively (Schmaljohn and Hooper, 2001; Schmaljohn et al., 1983). The L protein or RNA dependent RNA polymerase (RdRp) mediates both the replication of the genomic and anti-genomic viral RNAs and the transcription of viral mRNAs in the cytoplasm. The conservation of function across RNA polymerases suggests that broad spectrum nucleoside antivirals may be identified that act across genera in the *Bunyaviridae*.

Nucleoside analogs have been identified that acted on several members of the *Bunyaviridae*, albeit with differential levels of activity (Sidwell et al., 1972). The driving mechanism(s) underlying one of these drugs, RBV, has been difficult to capture primarily due to its ability to interact with both host and viral targets. For example, RBVs' activity against HTNV did not correlate with inhibition of inosine monophosphate dehydrogenase (IMPDH), but rather with production of RBV triphosphate (RBV-TP) (Sun et al., 2007) and an increase in mutation frequency (Severson et al., 2003). We hypothesized that the increase in resulting mutation frequency is due to the incorporation of RBV by the L protein into the viral RNAs (Severson et al., 2003). These findings led us to explore chemical modifications that would increase selectivity and activity of RBV-based scaffolds toward the L protein.

Focusing on the heterocyclic-β-riboside structure, we prepared a diverse series of 3-substituted 1,2,4-triazole-β-ribosides, including isosteric derivatives of RBV and linkage isomers that exhibit altered hydrogen-bonding capacity. We have previously evaluated representative compounds from this series as substrates for adenosine kinase (Kumarapperuma et al., 2007). Herein, we describe the antiviral activity of 1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR, Fig. 1) against 4 viruses, HTNV, ANDV, CCHFV, and RVFV. ETAR showed promising antiviral activity against HTNV, ANDV, and CCHFV, but not RVFV. Furthermore, it protected suckling mice from infection with HTNV to a degree that was similar to that seen with RBV.

2. Methods and materials

2.1. Chemistry and synthesis

The synthetic approach for the preparation of ETAR is shown in Scheme 1. Deacetylation of commercially available 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxylic acid, methyl ester **1** with $NaOCH_3$ (72%), protection with *tert*-butyldimethylsilyl chloride (TBSCl) (67%), followed by selective reduction of the ester with 2.5 equivalents of diisobutylaluminium hydride (DIBAL-H) gave the triazole aldehyde **2** (75%). The aldehyde was converted to the alkyne with Bestmann's reagent (78%) (Goundry et al., 2003). The TBS groups were removed with tetrabutylammonium fluoride (TBAF) and the product was recrystallized from 5% CH_3OH in dichloromethane to obtain pure ETAR as a crystalline powder (90%). Spectroscopic and mass spectrometric characterization data for ETAR are provided.[1] Com-

[1] ETAR compound characterization: mp 174–175 °C; FT-IR peaks (cm^{-1}) 2130. 1H NMR (200 MHz, CD_3OD) δ 8.72 (s, 1H), 5.84 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1′), 4.43 (m, 1H, H-2′), 4.29 (m, 1H, H-3′), 4.09 (m, 1H, H-4′), 3.83–3.79 (dd, 1H, $J_{5'a,5'b}$ = 12.3 and $J_{5'a,4'}$ = 3.3 Hz, H-5a), 3.73 (s, 1H), 3.70–3.65 (dd, 1H, $J_{5'b,5'a}$ = 12.9 and $J_{5'b,4'}$ = 4.7 Hz,