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Dual inhibition of HCV and HIV by ring-expanded nucleosides containing the 5:7-fused imidazo[4,5-*e*][1,3]diazepine ring system. In vitro results and implications

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ABSTRACT

Examples of ring-expanded nucleosides (RENs), represented by general structures **1** and **2**, exhibited dual anti-HCV and anti-HIV activities in both cell culture systems and the respective target enzyme assays, including HCV NTPase/helicase and human RNA helicase DDX3. Since HCV is a leading co-infection in late stage HIV AIDS patients, often leading to liver cirrhosis and death, the observed dual inhibition of HCV and HIV by the target nucleoside analogues has potentially beneficial implications in treating HIV patients infected with HCV.

Highly active antiretroviral therapy (HAART), employing a three-drug regimen acting on different stages of the viral life cycle, has dramatically increased the survival rate of the HIV-infected individuals, and has transformed Acquired Immunodeficiency Syndrome (AIDS) into a controllable chronic illness.[1,2] A fateful outcome of the chronic HIV condition, however, is the progressively weakening immune system since HIV primarily infects the CD4 lymphocytes which help the body fight infections.[3–5] This makes HIV patients vulnerable to opportunistic co-infections, including but not limited to that caused by the Hepatitis C virus (HCV).[6–9] The end-stage liver diseases caused by hepatitis viral infection is now one of the major causes of death (>50%) in HIV patients in the Western World.[10–12] In a recent study exploring the cause of death in HIV patients, a vast majority of the dead had tested positive for antibodies to HCV.[13] Out of the HIV opportunistic infections, HCV in particular has lately taken the center stage, causing alarms in the AIDS research community for many reasons, including (a) the vastly successful HAART therapy is considerably less effective with HIV patients co-infected with HCV,[14] (b) the protease inhibitors used in the HAART therapy exert a significant degree of extra strain on the liver that is already stressed by HCV.[14,15] This results in dramatic exacerbation of HCV and its accelerated progress to liver cirrhosis and death. Thus, patients on HAART therapy are even at more risk for liver diseases,[14,15] (c) the approved anti-HCV therapy with a combination of α-interferon and ribavirin was shown to decrease the potency of anti-HIV therapy because of the suspected molecular interaction of ribavirin with the reverse transcriptase inhibitors used in HAART, resulting in the latter's diminished effectiveness.[16] It is also not yet clear how the recently approved protease inhibitors for HCV treatment, including Victrelis (boceprevir),[17] Incivek (telaprevir),[17] and Olysio (simeprevir)[17] would affect disease progression of HIV patients infected with HCV. For these reasons, mutually compatible anti-HCV and anti-HIV drugs are needed to combat HCV co-infection in HIV patients. These drugs should neither exacerbate the clinical manifestations of the

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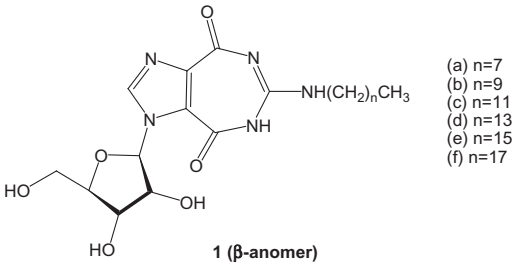
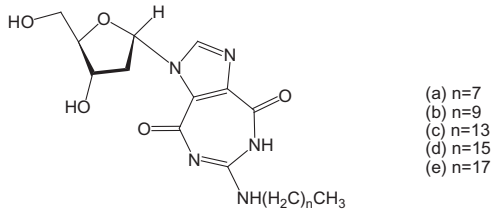
co-infection nor diminish the efficacy or effectiveness of the therapy used for treatment of the individual infection.

We have recently reported[18] the in vitro anti-HIV activity of a series of ring-expanded nucleosides (RENs) containing imidazo[4,5-*e*][1,3]diazepine ring system, represented by general structural formulas **1** and **2**. Out of the many compounds screened for inhibition of HIV-1 replication in virus-infected T cell line (MT4 cells), compounds **1f** and **2e** were identified as the lead compounds with low micromolar IC_{50}'s. Both compounds contained the long (18-carbon) alkyl chain at the C-6 position of the heterocycle, and differed from one another in their stereochemical configuration at the anomeric junction and the lack of 2′-OH group in **2e**. Neither compound was significantly toxic in ex vivo cell culture or in vivo in mice.[18] Both compounds strongly inhibited cellular RNA helicase DDX3, which the virus is believed to exploit for its replication as it lacked its own helicase.[19] Helicases are capable of unwinding duplex RNA and DNA structures by disrupting the hydrogen bonds that keep the two strands together.[20,21] This unwinding activity, which is normally accompanied by simultaneous hydrolysis of an NTP (ATP or GTP),[22] is essential for the virus replication.

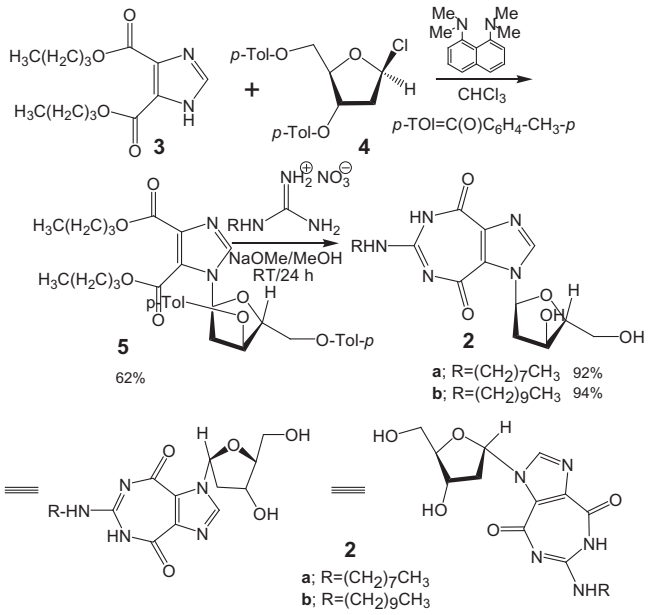
Several years ago, we had also reported[23] that a wide variety of RENs, containing both the imidazo[4,5-*e*][1,3]diazepine and imidazo[4,5-*e*][1,2,4]triazepine ring systems, potently inhibited the virus-encoded NTPases/helicases of several RNA viruses belonging to the Flaviviridae family, including but not limited to the West Nile Virus (WNV), Hepatitis C Virus (HCV), and the Japanese Encephalitis Virus (JEV). Subsequently, we had discovered[24] that compounds represented by general structural formulas **1** and **2** were especially effective in inhibition of the WNV NTPase/helicase. To our surprise and delight, these RENs failed to inhibit a truncated form of the human helicase $Suv3_{(\Delta 1-159)}$, which we had included in the study in order to assess their selectivity and toxicity.[24] Because of the perceived serious threat of WNV epidemic in North America in the early 2000's, our research efforts during that time, along with many other laboratories in US and Canada, were largely focused on WNV. In light of the promising results of **1** and **2** with HIV inhibition, coupled with the increasing problem of HCV co-infection in HIV patients as described above, and not to mention that HCV belongs to the same viral family as WNV, against which **1** and **2** had already exhibited potent antiviral activity, it was only logical to extend our investigation of these RENs to HCV, as elaborated in this Letter.



1 (β-anomer)



2 (α-anomer)



Scheme 1.

Syntheses of a majority of the compounds necessary for our present study, listed under general structural formulas **1** and **2**, were carried out by us earlier for the mentioned HIV and WNV studies[18,24] and so will not be repeated here. Synthesis of the two new compounds used in this study, **2a** and **2b**, is outlined in Scheme 1. Butyl imidazole-4,5-dicarboxylate (**3**) was glycosylated with 2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-erythropentofuranosyl chloride (**4**),[24] generated in situ from the corresponding α sugar, in the presence of *N*,*N*,*N*′,*N*′-tetramethyl-1,8-naphthalenediamine (proton sponge) to obtain butyl 1-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-α-D-erythropentofuranosyl)-4,5-imidazoledi-carboxylate (**5**) in 62% yield. Condensation of the latter with (*N*-octyl)- and (*N*-decyl)guanidinium nitrates, freshly generated from the reaction of 3,5-dimethylpyrazole-1-carboxamidine nitrate with *N*-octyl- and *N*-decylamine, respectively,[24] catalyzed by sodium methoxide in methanol, afforded **2a** and **2b** in 92% and 94%, respectively. Both compounds were fully characterized by spectroscopic and microanalytical data.[25]

The NTPase/helicase of HCV was expressed in *Escherichia coli* and purified by affinity chromatography.[25] The homogeneity of the enzyme preparation was verified by Coomassie Blue staining. In order to monitor the inhibitory potential of RENs toward helicase activity of HCV NTPase/helicase, radiolabelled partially double-stranded (ds) DNA was used as substrate.[25] Out of the 11 compounds listed under **1** and **2** that were biochemically screened, only **1f** and **2e** showed promising anti-helicase activity of HCV NTPase/helicase as shown in Table 1. This is remarkably consistent with what we had earlier observed with anti-HIV activity.[18] A long alky chain of a minimum of 18 carbon atoms, attached at the 6-position of the heterocycle, appears to be necessary for both anti-HIV and anti-HCV activities. We then repeated our above experiment of HCV NTPase/helicase with **1f** and **2e** using a double-stranded RNA substrate, and found that the compounds were equally effective as with a DNA substrate within a margin of experimental error. All other compounds listed under **1** and **2** failed to show activity even up to 300 μg/mL, although some of them (e.g., **1c** and **1d**) had earlier exhibited excellent activity against WNV NTPase/helicase.[24]

In light of the above encouraging anti-enzymic activity, both **1f** and **2e** were further screened for in vitro anti-viral activity against