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Flex-nucleoside analogues – Novel therapeutics against filoviruses

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ABSTRACT

Fleximers, a novel type of flexible nucleoside that have garnered attention due to their unprecedented activity against human coronaviruses, have now exhibited highly promising levels of activity against filoviruses. The Flex-nucleoside was the most potent against recombinant Ebola virus in Huh7 cells with an EC_{50} = 2 μM, while the McGuigan prodrug was most active against Sudan virus-infected HeLa cells with an EC_{50} of 7 μM.

Since the first reported fatal outbreak in the mid 1970s, members of the *Filoviridae* virus family, including the Ebola virus (EBOV), the Sudan virus (SUDV), and the Marburg virus (MARV), have continued to devastate many areas across the globe, with mortality rates as high as 90%.[1,2] One of the worst outbreaks of EBOV occurred in West Africa from 2013 to 2016, with over 28,000 documented infections and claiming more than 11,000 lives, including nearly 900 health care workers.[1] Filoviruses are a group of enveloped, single-stranded, negative-sense RNA viruses that cause fatigue, vomiting, and severe hemorrhagic fevers.[1,3,4] Members of the *Filoviridae* family are zoonotic viruses, where the primary reservoir is speculated to be fruit bats, however, it is unclear if this is the only reservoir or how the transmission to humans occurs.[2] The filoviruses are highly contagious and can easily spread through interaction with an infected individual by direct contact with bodily fluids including vomit, sweat, saliva, and respiratory secretions.[2,4] With the high potential for re-emergence of these lethal viruses, particularly due to "super-spreaders",[5,6] it is imperative that a viable treatment option be identified in order to better fight these crippling pathogens before the next outbreak occurs.

To date there are no available FDA approved treatments for filovirus infections. While various therapeutic options have been pursued including vaccines,[7] monoclonal antibodies,[4,8] and recombinant proteins,[9,10] many of these have yet to reach clinical trials and may ultimately not translate well to effective treatments that can be made readily available during an outbreak, particularly in suboptimal conditions.[11] One therapeutic option for the development of antiviral treatments is the use of nucleoside analogues. Nucleoside analogues have long been the cornerstone of antiviral therapies due to their ability to inhibit viral replication because they mimic the structure of the natural nucleosides.[12,13] As such, they can be recognized by cellular or viral enzymes, including the viral DNA or RNA polymerases. Moreover, because they contain various structural modifications, this leads to cessation of viral replication, typically due to chain termination.[13] Various nucleoside analogues against filoviruses such as EBOV have already been proposed including S-adenosylhomocysteine hydrolase (SAHase) inhibitors c3Ado and c3Nep (Fig. 1),[14,15] and the monophosphate derivative of BCX4430,[16] an adenosine analogue that acts as a non-obligate chain terminator, however, none of these have progressed to the clinic. Most recently GS-5734, a monophosphoramidate prodrug adenosine analogue which targets EBOV RNA-dependent RNA polymerase (RdRp), exhibited very potent activity against both EBOV and MARV,[17,18] further demonstrating the potential for finding effective nucleoside inhibitors of filoviruses.

Over the past several years, research in our laboratory has focused on the development of flexible nucleoside analogues, termed "Fleximers".[19–26] One type of fleximer features a purine ring that is "split" into its imidazole and pyrimidine moieties.

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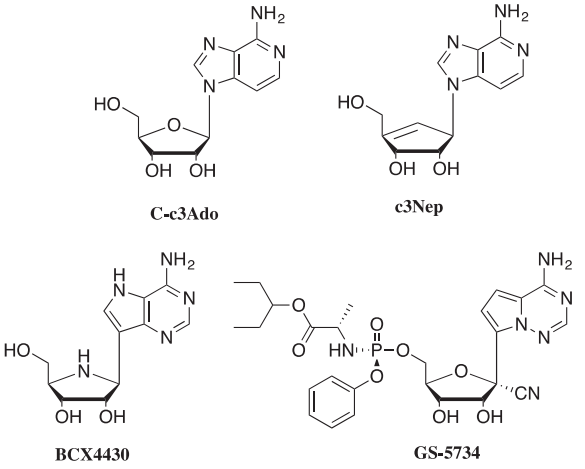


Fig. 1. Nucleoside based inhibitors with reported anti-Ebola activity.

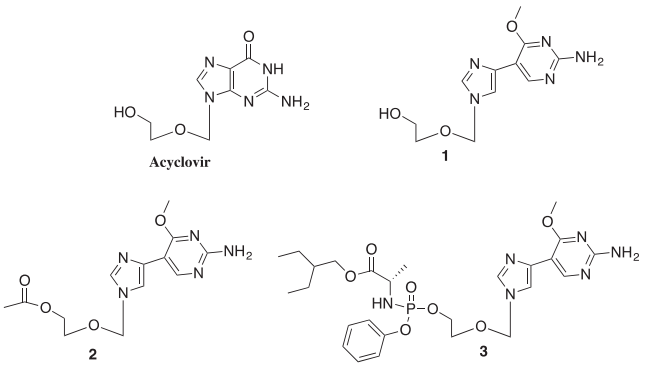


Fig. 2. Structure of Acyclovir and the target flexible nucleoside analogues.

The two pieces remain connected by a single C—C bond, thus introducing free rotation between the two heterocyclic components without losing the necessary groups needed for recognition (Fig. 2).[19,20] This strategic design retains the hydrogen bonding patterns needed for recognition, while allowing the Flex-nucleoside to interact with alternative binding moieties, such as different amino acids in the binding pocket, that were previously unattainable by the parent nucleoside.[19,20,25] Studies within our lab have also shown that their inherent flexibility allows for an increase in binding affinity compared to corresponding rigid inhibitors, as well as the ability to overcome point mutations in biologically relevant enzymatic binding sites, thus providing potential for overcoming the development of drug resistance.[19–25]

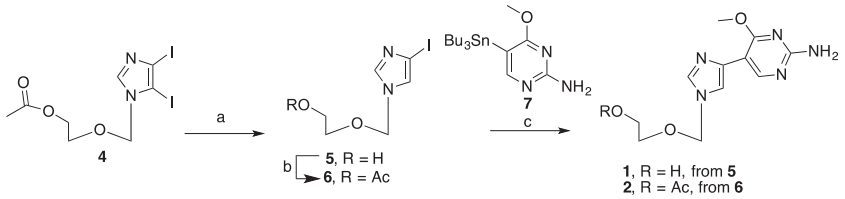
More importantly, recent work with some fleximer versions of the FDA-approved nucleoside Acyclovir, revealed significant activity against human coronaviruses Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV), representing the first nucleoside analogues to exhibit low micromolar levels of anti-CoV activity.[26] This was groundbreaking since nucleoside analogues had to that point failed to show viable levels of activity against these deadly viruses. As a result, this prompted further evaluation of the Flex-analogues against other viruses, particularly given the dual anti-CoV and anti-EBOV activity recently noted by GS-5734.[17] Herein, we report the anti-filovirus activity for these analogues, as well as the corresponding phosphoramidate prodrug **3** (Fig. 2).

The synthesis of the target compounds began with the substituted imidazole **4**, utilizing the routes previously employed in our group (Scheme 1).[26] Treatment with sodium sulfite in a 30% ethanol/water solution resulted in simultaneous deacetylation and selective deiodination to provide key intermediate **5**. Acetylation of **5** then generated **6**, the 5′ protected intermediate needed for the prodrug synthesis. In parallel, the organometallic coupling reagent **7** was synthesized starting from the commercially available 2-amino-4-methoxypyrimidine.[27,28] Stille coupling of **7** to **5** gave **1**. Alternatively, using the acetylated **6**, Stille coupling provided the desired double prodrug **2**.

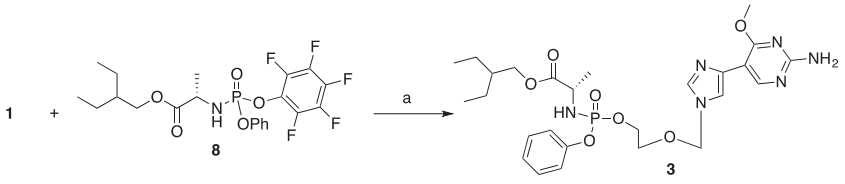
Synthesis of the McGuigan ProTide[29–33] started with commercially available L-alanine and utilized literature procedures to generate the phosphoramidate **8** (Scheme 2).[34] Reaction of **8** with fleximer **1** in the presence of *tert*-butyl magnesium chloride then provided the desired McGuigan ProTide **3** in 69% yield.

After the successful synthesis of the three Flex-analogues **1**, **2**, and **3**, the compounds were screened against a panel of filoviruses including EBOV, MARV, and SUDV, as well as other hemorrhagic fever viruses such as Lassa and Rift Valley Fever. The first series of assays utilized HeLa cells infected with live-virus isolates of EBOV (Makona), SUDV (Gulu), and MARV (Ci67). Activity against all three viruses was observed for the McGuigan prodrug **3**, with the best activity against SUDV (Table 1).

The second series of assays utilized Huh7 cells infected with recombinant reporter EBOV, Lassa, and Rift Valley Fever viruses. As observed in the first series of assays, compound **3** was active against EBOV at a similar concentration, however, compound **1**



Scheme 1. Reagents and conditions: (a) Na_2SO_3, 30% EtOH, 120 °C, 84%; (b) Ac_2O, NEt_3,DMAP, 97%; (c) Pd_2dba_3 $CHCl_3$, **5** or **6**, CuI, CsF, DMF, 50 °C, 20%.



Scheme 2. (a) ^{t}BuMgCl, THF, 69%.

Table 1
Antiviral activity of nucleoside analogues in infected HeLa cells, values are in μM.

CMPD	EBOV		SUDV		MARV	
	EC_{50}	CC_{50}	EC_{50}	CC_{50}	EC_{50}	CC_{50}
1	>100	>100	>100	>100	>100	>100
2	44 ± 13	>100	20 ± 10	>100	70 ± 27	>100
3	29 ± 9	>100	7 ± 2	>100	62 ± 13	>100

Table 2
Antiviral activity of nucleoside analogues against recombinant reporter viruses in Huh7 cells in μM.

CMPD	EBOV		Lassa Virus		Rift Valley Fever	
	EC_{50}	CC_{50}	EC_{50}	CC_{50}	EC_{50}	CC_{50}
1	2.2 ± 0.3	>50	>50	>50	>50	>50
3	27.2 ± 2.2	>50	>50	>50	>50	>50

exhibited the best activity (EC_{50} = 2.2 ± 0.3 μM) against EBOV in Huh7 cells (Table 2).

Infectious diseases such as EBOV continue to pose a serious health threat due to the high mortality rates associated with these deadly viruses. While ongoing studies have identified various therapeutics as potential EBOV treatments, there are currently no FDA approved vaccines or therapeutics, and as such, it is imperative that an effective treatment option is developed. Within this study we found that both compounds **1** and **3** exhibited antiviral activity against a recombinant reporter EBOV in Huh7 cells, though surprisingly the McGuigan prodrug was ~10-fold less potent (EC_{50} = 2.2 ± 0.3 μM and 27.2 ± 2.2 μM respectively). Against wild-type viruses in HeLa cells, compound **1** had no detectable activity, though compound **3** inhibited both EBOV and SUDV (EC_{50} = 29 ± 9 and 7 ± 2 μM respectively). The difference in activity of **1** in the Huh7 cells compared to the HeLa cells is most likely due to a difference in specific metabolism of the compound in those cells lines, however, further studies are needed to confirm this hypothesis. Efforts are currently underway to better understand the mechanism of action of these compounds and how they might interact with the viral RdRp or other viral replication enzymes. The results of those studies will be reported as they become available.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.04.069.

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