Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Flex-nucleoside analogues – Novel therapeutics against filoviruses

Mary K. Yates a, Mithun R. Raje a, Payel Chatterjee b, Christina F. Spiropoulou b, Sina Bavari c, Mike Flint b, Veronica Soloveva c, Katherine L. Seley-Radtke a,⇑

a Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD 21250, United States
b Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA 30329, United States
c US Army Medical Research Institute, Frederick, MD 21702, United States

Article info
Article history:
Received 26 March 2017
Revised 21 April 2017
Accepted 21 April 2017
Available online 22 April 2017

Keywords:
Nucleoside
Filovirus
Ebola
Antiviral
Fleximers

A B S T R A C T
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 EC50 = 2 µM, while the McGuigan prodrug was most active against Sudan virus-infected HeLa cells with an EC50 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 FDA approved treatments for filovirus infections. While various therapeutic options have been pursued including vaccines,7 monoclonal antibodies,7,8 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 monophosphate 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.
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). 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. 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.

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. 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. Herein, we report the anti-filovirus activity for these analogues, as well as the corresponding phosphoramidate prodrug (Fig. 2).

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

Synthesis of the McGuigan ProTide started with commercially available l-alanine and utilized literature procedures to generate the phosphoramidate (Scheme 2). Reaction of with fleximer in the presence of tert-butyl magnesium chloride then provided the desired McGuigan ProTide in 69% yield.

After the successful synthesis of the three Flex-analogues, 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 (Cie67). Activity against all three viruses was observed for the McGuigan prodrug , 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 was active against EBOV at a similar concentration, however, compound

![Fig. 1. Nucleoside based inhibitors with reported anti-Ebola activity.](image1)

![Fig. 2. Structure of Acyclovir and the target flexible nucleoside analogues.](image2)

![Scheme 1. Reagents and conditions: (a) Na2SO3, 30% EtOH, 120 °C, 84%; (b) Ac2O, NEt3,DMAP, 97%; (c) Pd2dba3 CHCl3, 5 or 6, Cul, CsF, DMF, 50 °C, 20%.](scheme1)

![Scheme 2. (a) tBuMgCl, THF, 69%.](scheme2)
exhibited the best activity (EC₅₀ = 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 (EC₅₀ = 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 (EC₅₀ = 27.2 ± 9 μM and 29 ± 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.

Acknowledgments

This work was supported by the National Institutes of Health-T32 GM066706 and R21 AI097685 (KSR). We would also like to thank USAMRIID/MTS colleagues Dima Gharaibeh, Ylenia Cau, Tara Kenny, Cary Retterer, Rouzbeh Zamani, Glenna Gomba and Collin Dube for assistance. Work at USAMRIID was funded by The Joint Science and Technology Office for Chemical and Biological Defense (JSTO-CBD) of the Defense Threat Reduction Agency (DTRA). Opinions, interpretations, conclusions, and recommendations reported herein are those of the author and are not necessarily endorsed by the U.S. Army. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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.

Table 1

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

| CMPD | EBOV EC₅₀ | CC₅₀ | SUDV EC₅₀ | CC₅₀ | MARV EC₅₀ | CC₅₀ |
|------|-----------|------|-----------|------|-----------|------|
| 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 EC₅₀ | CC₅₀ | Lassa Virus EC₅₀ | CC₅₀ | Rift Valley Fever EC₅₀ | CC₅₀ |
|------|-----------|------|------------------|------|------------------------|------|
| 1    | 2.2 ± 0.3 | >50  | >50              | >50  | >50                    | >50  |
| 3    | 27.2 ± 2.2| >50  | >50              | >50  | >50                    | >50  |

References

1. Boisen ML, Hartnett JN, Goba A, et al. Annu Rev Virol. 2016;3:147–171.
2. Moghadam SRJ, Omidi N, Bayrami S, Moghadam SJ, Seyed Alanaghi S. Asian Pac J Trop Biomed. 2015;5:260–267.
3. Rongeron V, Feldmann H, Gruber G, Becker S, Leroy EM. J Clin Virol. 2015;64:111–119.
4. Mendoza EJ, Qui X, Koberger GP. Trends Mol Med. 2016;22:164–173.
5. Lau MS, Dalziel BD, Funk S, et al. Proc Natl Acad Sci USA. 2017;114:2337–2342.
6. L. H. Sun. Disease ‘superspreaders’ accounted for nearly two-thirds of Ebola cases, study finds, www.washingtonpost.com/news/to-your-health/wp/2017/02/13/disease-superspreaders-accounted-for-nearly-two-thirds-of-ebola-cases-study-finds/?utm_term=55a3a8cb28a, (accessed March 20, 2017).
7. Mire CE, Geisbert TW, Feldmann H, Marzi A. Expert Rev Vaccines. 2016;15:1421–1430.
8. Qiu X, Audet J, Wong G, et al. Sci Transl Med. 2012;4:138ra181.
9. Geisbert TW, Hensley LE, Jahrling PB, et al. Lancet. 2003;362:1953–1958.
10. Geisbert TW, Lee AC, Robbins M, et al. Lancet. 2010;375:1986–1995.
11. Picazo E, Gironzetto F. Drug Discov Today. 2015;20:277–286.
12. De Clercq E, Neyts J. Nat Rev Microbiol. 2004;2:704–720.
13. De Clercq E, Neyts J. Handb Exp Pharmacol. 2009;53–84.
14. Huggins JW, Zhang ZX, Monath TP. Antiviral Res. 1991;15.
15. Coulombe RA, Sharma RP, Huggins JW. Eur J Drug Metab Pharmacokinet. 1995;20:197–202.
16. Warren TK, Wells J, Paschal RG, et al. Nature. 2014;508:402–405.
17. Warren TK, Jordan R, Lu MK, et al. Nature. 2016;531:381–385.
18. Lo MK, Jordan R, Arvey A, et al. Sci Rep. 2017;7:43395.
19. Seley KL, Zhang L, Hagos A. Org Lett. 2001;3:3209–3210.
20. Seley KL, Zhang L, Hagos A, Quirk S. J Org Chem. 2002;67:3365–3373.
21. Seley KL, Quirk S, Salim S, Zhang L, Hagos A. Bioorg Med Chem Lett. 2003;13:1985–1988.
22. Polak M, Seley KL, Havel J. J Am Chem Soc. 2004;126:8159–8168.
23. Quirk S, Seley KL. Biochemistry. 2005;44:13172–13178.
24. Quirk S, Seley KL. Biochemistry. 2005;44:10854–10863.
25. Seley KL, Salim S, Zhang L, O’Daniel PJ. J Org Chem. 2005;70:1612–1619.
26. Peters HL, Jochmans D, de Wilde AH, et al. Bioorg Med Chem Lett. 2015;25:2923–2926.
27. Minkawa N, Kojima N, Hikishima S, et al. J Am Chem Soc. 2003;125:9970–9982.
28. Wauchope OR, Velasquez M, Seley-Radtke K. Synthesis (Stuttg). 2012;44:3496–3504.
29. Mehellou Y, Balzarini J, McGuigan C. ChemMedChem. 2009;4:1779–1791.
30. Pertussai F, Serpi M, McGuigan C, Antivir Chem Chemother. 2012;22:181–203.
31. Medala K, McGuigan C, Future Med Chem. 2012;4:625–650.
32. Cahard D, McGuigan C, Balzarini J. Mini Rev Med Chem. 2004;4:371–374.
33. Pradere U, Garnier-Amblard EC, Coats SJ, Amblard F, Schinazi RF. Chem Rev. 2014;114:9154–9218.
34. Ross BS, Reddy PG, Zhang HR, Rachakonda S, Sofia MJ. J Org Chem. 2011;76:8311–8315.