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Minireview

New Nucleoside Analogues for the Treatment of Hemorrhagic Fever Virus Infections

Erik De Clercq*[a]

3-Deazaneplanocin A

Galidesivir

GS-6620

Remdesivir

Ribavirin

EICAR

Pyrazofurin

Favipiravir

Hemorrhagic fever virus (HFV) infections (i.e. Ebola)
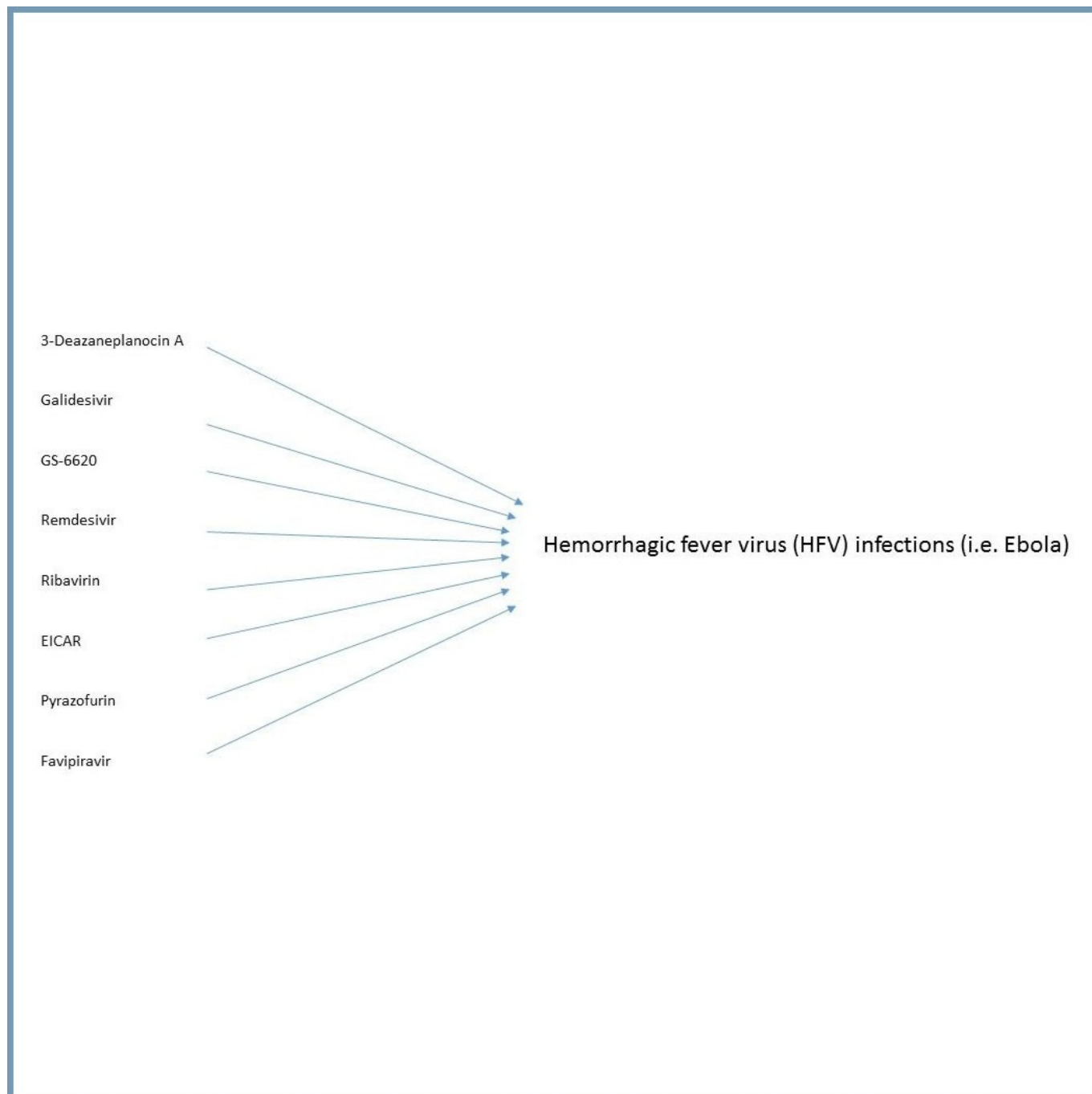
Abstract: Eight different compounds, all nucleoside analogues, could presently be considered as potential drug candidates for the treatment of Ebola virus (EBOV) and/or other hemorrhagic fever virus (HFV) infections. They can be considered as either (i) adenine analogues (3-deazaneplanocin A, galidesivir, GS-6620 and remdesivir) or (ii) guanine analogues containing the carboxamide entity (ribavirin, EICAR, pyrazofurin and favipiravir). All eight owe their mechanism of action to hydrogen bonded base pairing with either (i) uracil or (ii) cytosine. Four out of the eight compounds (galidesivir, GS-6620, remdesivir and pyrazofurin) are C-nucleosides, and two of them (GS-6620, remdesivir) also contain a phosphoramidate part. The C-nucleoside and phosphoramidate (and for the adenine analogues the 1′-cyano group as well) may be considered as essential attributes for their antiviral activity.

The hemorrhagic fever virus (HFV) infections, including Lassa and Ebola, that we highlighted as potential targets for antiviral agents in 1993[1] have essentially remained unchanged, and now, 27 years later, we are still awaiting the first antiviral drug(s) to be formally approved for the treatment of HFV infections. The first antiviral compound ever to be reported to be effective in the treatment of any HFV infection, that is, Lassa, that could be used at any point in the illness as well as for postexposure prophylaxis, was ribavirin.[2] Ribavirin (Virazole, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (Figure 1) was the first synthetic nucleoside analogue ever reported (in 1972) to be active against a broad spectrum of both RNA and DNA viruses.[3] In their final remarks, Sidwell et al. (1972) stated, rightfully that "the antiviral spectrum of Virazole was the broadest ever reported for any synthetic material that does not induce interferon", although the eventual clinical use of Virazole would eventually be limited to RNA virus infections.[4] One of the structural analogues mentioned in the latter article was EICAR (5-ethynyl-1-β-D-ribofuranosyl-imidazole-4-carboxamide) (Figure 1).

EICAR had originally been reported as an antileukemic agent in mice.[5] In its antiviral activity, it showed a similar spectrum as ribavirin, being active against pox-, toga-, arena-, reo-, orthomyxo- and paramyxovirus infections, with a potency that was 10- to 100-fold greater than that of ribavirin.[6] As originally ascertained for ribavirin,[7] EICAR also proved to be a potent inhibitor of IMP dehydrogenase, thus blocking the biosynthesis of GMP.[8] The depletion of the intracellular GTP and dGTP pools, resulting from the decreased biosynthesis of GMP, may also explain various other effects of EICAR, such as its potentiating effect on the anti-HIV activity of ddI (2′,3′-dideoxyinosine)[9] and its inhibitory effects on the replication of a variety of viruses such as the double-stranded RNA virus, IPNV (infectious pancreatic necrosis virus).[10,11] In the latter publication, yet another antiviral compound, pyrazofurin, was mentioned to be a specific inhibitor or IPNV.[11]
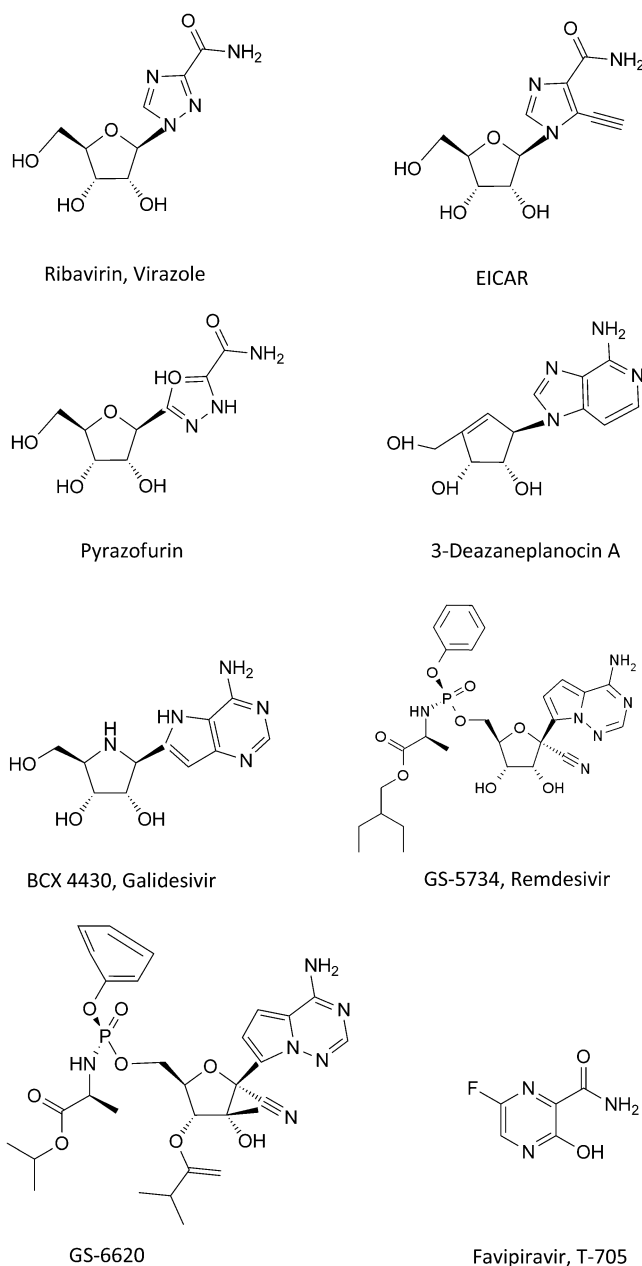

Figure 1. Chemical structures.

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Pyrazofurin [3-(β-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide] (Figure 1) is an inhibitor of OMP decarboxylase, a key enzyme in the de novo pyrimidine mononucleotide biosynthesis: it has proven to be active against both (+)RNA viruses [picorna (polio, Coxsackie B4), toga (sindbis) and flavi (yellow fever)] and (−)RNA viruses [paramyxo (measles, RSV), orthomyxo (influenza), rhabdo (VSV) and arena (Junin, Tacaribe)].[4,12] Pyrazofurin has been found to be an exquisitely potent inhibitor of VSV (vesicular stomatitis virus), which belongs to a family (rhabdoviridae), closely related to the family of the filoviridae to which Ebola virus (EBOV) and Marburg virus belong. VSV can be handled in conventional safety conditions, whereas EBOV and Marburg virus require biosafety level 4; VSV could therefore be recommended as a paradigm for predicting antiviral activity against EBOV.[13] Pyrazofurin has so far not been evaluated for its potential activity against EBOV; it should be done so, as has already been done with the neplanocin A analogues. These analogues are known to be targeted at the S-adenosylhomocysteine hydrolase.[14] The prototype of this class of compounds is 3-deazaneplanocin A (Figure 1). Two decades ago, 3-deazaneplanocin A was shown to be effective against a lethal EBOV infection in mice.[15,16] The compound induced massively increased interferon-α production in EBOV-infected mice,[16] a startling observation that was never followed up.

When reviewing in 2015 possible therapeutic strategies to block EBOV infections, I mentioned 3-deazaneplanocin A, besides BCX4430 and T-705 (favipiravir).[17] Later added to the list were GS-5734 (remdesivir) and ZMapp.[18] The response to ZMapp, a mixture of three monoclonal antibodies directed against the surface glycoprotein of EBOV, was beneficial but did not meet the prespecified statistical threshold for efficacy [death occurred in 13 of 35 patients (37%) who received the current standard of care alone as compared to 8 of 36 patients (22%) who received the current standard of care plus ZMapp].[19] Apparently the outbreak of Ebola in 2014–2016 ended before any incontrovertible evidence of any intervention could be assessed.

In this review I will focus on the potential of favipiravir (T-705), BCX4430 (Galidesivir) and GS-5734 (Remdesivir) on the treatment of EBOV and other HFV infections.

The imino-C-nucleoside BCX4430 (Galidesivir) was first reported by Warren et al.[20] to be active against a wide range of viruses, including filo-, toga-, bunya-, arena-, paramyxo, corona-, flavi-, orthomyxo- and picornaviruses. It was found to completely protect cynomolgus macaques from Marburg virus infection when administered as late as 48 hours after infection.[20] It effectively blocked yellow fever virus infection in a hamster model.[21] It is now under development for the treatment of EBOV infection.[22] BCX4430 (Galidesivir) (Figure 1) has also been found effective against Zika virus in cell culture and in a lethal mouse model.[23] Antiviral activity of BCX4430 has also been reported against West Nile virus, a typical mosquito-transmitted flavivirus, and against tick-borne flaviviruses, Kyasanur Forest disease virus (KFDV).[24] It inhibits infection of Rift Valley fever virus (RVFV), a mosquito-borne pathogen that causes severe disease in humans and livestock in sub-Saharan

Africa and the Arabian Peninsula, in Syrian golden hamsters.[25] The phosphoramidate prodrug of the pyrrolo[2,1-f][triazin-4-amino] adenine C-nucleoside GS-5734 (Remdesivir) (Figure 1) was first reported to protect 100% of EBOV-infected rhesus monkeys against a lethal EBOV infection.[26] It was also found active against other emerging viruses such as respiratory syncytial virus (RSV) and hepatitis C virus (HCV), and the presence of the 1′-cyano group in remdesivir was found to be critical in providing selectivity toward the viral (RNA) polymerases.[27] GS-5734 was then found to be highly inhibitory to various viruses other than filo, that is, pneumo-, paramyxo- and coronaviruses.[28] That GS-5734 inhibited both epidemic and zoonotic coronaviruses, and might prove effective against emerging coronaviruses in the future was emphasized by Sheahan et al.[29] The viral RNA polymerase and the proofreading exoribonuclease were suggested as being responsible for the coronavirus susceptibility to remdesivir.[30] The viral RNA polymerase has also been identified as the target enzyme of paramyxoviruses (i.e. Nipah virus) for the antiviral activity of GS-5734.[31] For both EBOV RNA-dependent RNA polymerase (RdRp) and RSV RdRp, chain termination was delayed and predominantly seen at position $i+1$.[32] The first newborn baby to have survived congenital EBOV infection, received remdesivir in addition to ZMapp and a buffy coat transfusion from an Ebola survivor.[33] Favipiravir (T-705) (Figure 1) is a pyrazine derivative, that is, 6-fluoro-3-hydroxy-2-pyrazinecarboxamide, sharing a common structural feature, that is a carboxamide entity, with ribavirin, EICAR and pyrazofurin, and which is also an essential (hydrogen bonding) part of guanine (-HN$_1$-C$_6$O-).[34,35] As already mentioned, ribavirin and EICAR would primarily owe their antiviral activity to interference with the IMP dehydrogenase, a key enzyme in the biosynthesis of GMP and GTP. Yet, Eriksson et al.[36] reported inhibition of influenza virus RNA polymerase by ribavirin triphosphate, and when Furuta et al.[37] had revealed the in vitro and in vivo activities of T-705 against influenza virus, they attributed the mode of action of T-705 to a specific inhibition of the influenza virus RNA polymerase by the T-705 RTP (ribofuranosyl triphosphate).[38] To this end (Scheme 1), T-705 had first to be converted to its ribofuranosyl monophosphate (RMP) by a

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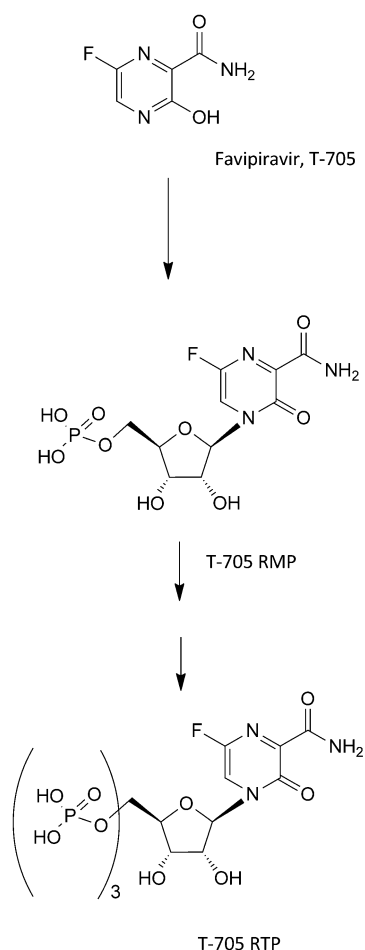
Scheme 1. Metabolism of T-705.

phosphoribosyl transferase, and then further processed by kinase(s) to its diphosphate (RDP) and triphosphate (RTP), before the latter would interact in a GTP-competitive manner with the viral RNA synthesis.

That T-705 (Favipiravir), as a viral RNA polymerase inhibitor, offers great potential in the treatment of a wide variety of RNA virus infections, has been reviewed repeatedly.[39–42] Sidwell et al.[43] demonstrated that T-705 inhibited a lethal avian influenza A (H5N1) virus infection in mice. This was confirmed by Kiso et al..[44] It was ascertained that in the influenza A (H5N1) virus-infected cells T-705 was metabolized to T-705 RTP.[45] The latter is incorporated into the nascent RNA strand as a purine nucleotide analog (reminiscent of GMP) and inhibits strand extension.[46] The RTP of T-705 acts as a GTP-competitive inhibitor of the influenza viral RNA polymerase.[47] Favipiravir inhibits in vitro replication of a wide range of influenza viruses, including those resistant to currently available drugs[48] and those isolated from patients who had previously received favipiravir.[49] Favipiravir has been approved, as Avigan, in Japan for the treatment of influenza.[50]

Lethal mutagenesis has been proposed to be a key mechanism in the activity of T-705 (Favipiravir) against influenza A (H1N1) viruses in vitro.[51] Lethal mutagenesis has also been suggested for the activity of favipiravir against norovirus repli-

cation[52] and hepatitis C virus.[53] Such mechanism of antiviral activity was originally proposed for ribavirin.[54, 55] However, the activity of ribavirin against yellow fever virus could not be explained by an error-prone mechanism.[56]

Favipiravir has proven effective against virtually all hemorrhagic fever virus infections: arena- and bunyaviridae,[57] Pichinde virus (an arenavirus),[58, 59] Lassa fever virus,[60] yellow fever virus,[61] Western Equine encephalitis virus,[62] West Nile virus,[63] Punta Toro, a phlebovirus,[64] the hantavirus Maporal,[65] Rift Valley Fever virus [Phlebovirus (Bunyaviridae)],[66] Crimean-Congo hemorrhagic fever virus [Nairovirus (Bunyaviridae)],[67] Severe Fever with Thrombocytopenia Syndrome virus (SFTSV), a recently identified emerging virus,[68] and norovirus (Caliciviridae).[69, 70] Resistance to T-705 has never been reported, except for Chikungunya virus (Alphaviridae) which acquired resistance due to the K291R mutation in the RNA-dependent RNA polymerase (RdRp).[71]
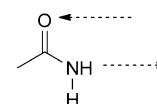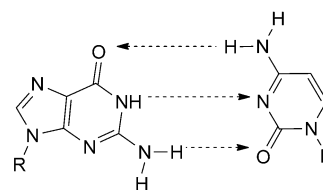
Favipiravir proved successful in the treatment of EBOV infection in mice.[72, 73] In nonhuman primates, treated intravenously with favipiravir, five of six animals (83%) survived a lethal Marburg virus infection.[74] In patients with the EBOV infection in

Adenine - Uracil pairing
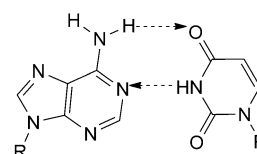
3-Deazaneplanocin A
Galidesivir
Remdesivir
GS-6620

Guanine - Cytosine pairing

carboxamide

Ribavirin
EICAR
Pyrazofurin
Favipiravir

Figure 2. Hydrogen bonding.

Sierra Leone, favipiravir was found to increase the survival rate from 35.3% (30/85) to 56.4% (22/39).[75] Another study carried out in Guinea ended with the statement that favipiravir monotherapy merits further study in patients with medium to high viremia but not in those with very high viremia.[76] Guedj et al.[77] concluded that favipiravir may have a potential role at high doses in the treatment of EBOV infections in humans.

Taking into account that rhabdo- and filoviruses are closely related, it is not surprising that favipiravir has also been advocated for the postexposure prophylaxis of rabiesvirus, as a potential alternative to rabies immunoglobulin.[78]

The compounds described here can be considered as either adenine derivatives (3-deazaneplanocin A, galidesivir, remdesivir or guanine derivatives (ribavirin), EICAR, pyrazofurin, favipiravir). This is immediately obvious for the adenine derivatives, which can be expected to interfere with viral RNA synthesis at the level of the RdRp (RNA-dependent RNA polymerase), where they can serve as competitive inhibitors with respect to ATP (based on the hydrogen bonds formed between adenine and uracil) (Figure 2). In addition, the adenine analogues can also serve as S-adenosylhomocysteine (SAH) hydrolase inhibitors, thus interfering with the S-adenosylmethionine (SAM)-dependent methylation reactions.

That ribavirin, EICAR, pyrazofurin and favipiravir would be able to act as guanine derivatives thus competing with GTP at the RdRp level is less obvious. Yet, all 4 compounds share a carboxamide group, reminiscent of the carboxamide ($-C_{(6)}O-N_{(1)}H-$) part of guanine, which could explain the hydrogen bonding with cytosine (Figure 2). In addition to their action at the RdRp level, the carboxamide-containing derivatives could also interfere with the IMP dehydrogenase activity, thus suppressing the biosynthesis of GTP, as has been specifically demonstrated for ribavirin and EICAR.

The exact mechanism of action of favipiravir remains to be assessed; it may well vary from one virus to another. As far as the activity of favipiravir RTP against influenza A virus polymerase is concerned, this has been ascribed to "ambiguous base-pairing".[79]

In 2016, I proposed that "C-nucleosides should be revisited".[80] This proposal was prompted by the advent of two new C-nucleosides, BCX4430 (Figure 1), which has in the meantime, been further pursued (as galidesivir) for the treatment of EBOV infections (see *supra*), and GS-6620 (compound 2, containing a 2′-C-methyl group imparting specific activity against hepatitis C virus (GCV).[80] GS-6620 (Figure 1) also contained a 1′-cyano group, which later on was found to be critical in providing selectivity toward viral (RNA) polymerases.[27] GS-6620 has only been reported for its potential as an anti-HCV agent.[81–83] It has not been thoroughly explored for its potential activity against EBOV or any other hemorrhagic fever virus (HFV) infections. According to literature data,[27] it would only have weak activity against EBOV.

Suggestions for further chemical synthesis

Remdesivir (GS-5734) is at present a leading drug candidate for the treatment of EBOV infections. Its chemical attributes are that it is a C-nucleoside, extended by a phosphoramidate and equipped with a CN group. Galidesivir (BCX4430) is also a C-nucleoside, but what would happen if it would also contain a CN group and would be converted to a phosphoramidate prodrug? For favipiravir, its *N*-nucleoside T-1106 is more effective than the free 2-pyrazinecarboxamide against yellow fever virus infection in hamsters,[61] but less so against Punta Toro virus in mice.[64] What would happen if T-1106 would be converted to its C-nucleoside, and, furthermore, extended by a phosphora-
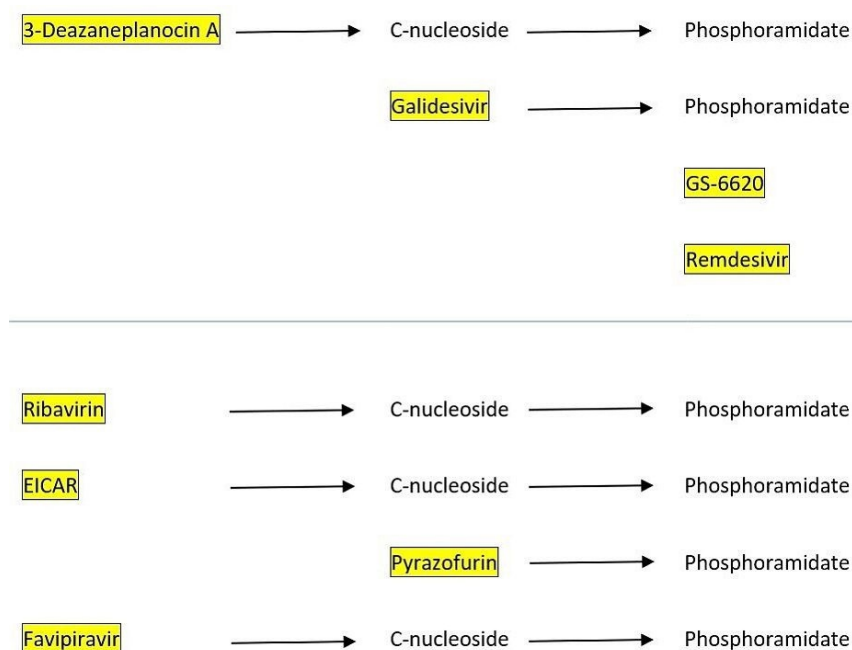
Scheme 2. (Candidate) antiviral compounds against EBOV.

midate entity? Also, the forgotten C-nucleoside, pyrazofurin,[80] could be converted to its phosphoramidate, and EICAR, which contains an ethynyl, reminiscent of the cyano group, could be first transformed to its C-nucleoside before being converted to its phosphoramidate (Scheme 2). Any of these chemical interventions may provide potential clues in the design of new medicines against EBOV and other HFV infections.

Conclusions

The (candidate) antiviral compounds currently available for the treatment of EBOV and other HFV infections are 3-deazaneplanocin A, galidesivir, GS-6620, remdesivir, ribavirin, EICAR, pyrazofurin and favipiravir. They are boxed in (Scheme 2).

In attempts to increase their efficacy and potentially lower their toxicity, they could, where applicable, first be converted to their C-nucleoside, and, subsequently, to their phosphoramidate (Scheme 2). For GS-6620 and Remdesivir, which, in addition, also contain a cyanogroup, these manipulations have already been achieved.

Conflict of interest

The author declares no conflict of interest.

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