

Vesicular stomatitis virus (VSV) as a paradigm for predicting antiviral activity against Ebola virus (EBOV)

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ABSTRACT

There are at present no antivirals available which have been formally licensed for clinical use for the treatment of Ebola virus (EBOV) infections in humans. The most advanced to be approved for this purpose is favipiravir (T-705), a viral RNA polymerase inhibitor. Under consideration are BCX4430, also a viral RNA polymerase inhibitor, and 3-deazaneplanocin A and various other S-adenosylhomocysteine (SAH) hydrolase inhibitors. A number of compounds which have been approved for other purposes seem to interact with the cell entry of

EBOV. Some compounds like pyrazofurin have been found to be exquisitely potent inhibitors of vesicular stomatitis virus (VSV). VSV belongs to the *rhabdoviridae*, a family closely related to the family of the *filoviridae* to which EBOV and Marburg virus belong. VSV, unlike EBOV and Marburg virus which require biosafety level 4, can be handled in conventional safety conditions.

Key words: Ebola virus (EBOV); vesicular stomatitis virus (VSV); *rhabdoviridae*; *filoviridae*; favipiravir; BCX4430; pyrazofurin; SAH hydrolase

INTRODUCTION

Interferon was originally considered as the panacea for the treatment of virus infections, just as antibiotics and particularly penicillin had proved to be the case for bacterial infections. Initially, there were two approaches for the clinical use of interferon: exogenous and endogenous. Exogenous use of interferon was originally not very practical because of the limited amounts of exogenous interferon available. Thus, the main emphasis was put on the endogenous induction of interferon, and viruses would initially seem as the best choice for this purpose (as after all, Isaacs and Lindenmann had discovered interferon) (1) by using influenza virus as the inducer. To verify whether the induced antiviral substance was interferon, an assay system had to be developed that could quantitate the amount of interferon induced by any putative inducer, and for this titration, a challenge virus had to be used which in the early interferon days was either vaccinia virus (VV) or vesicular stomatitis virus (VSV). The first belongs to the *poxviridae* (with variola virus (smallpox) as the prototype), the second

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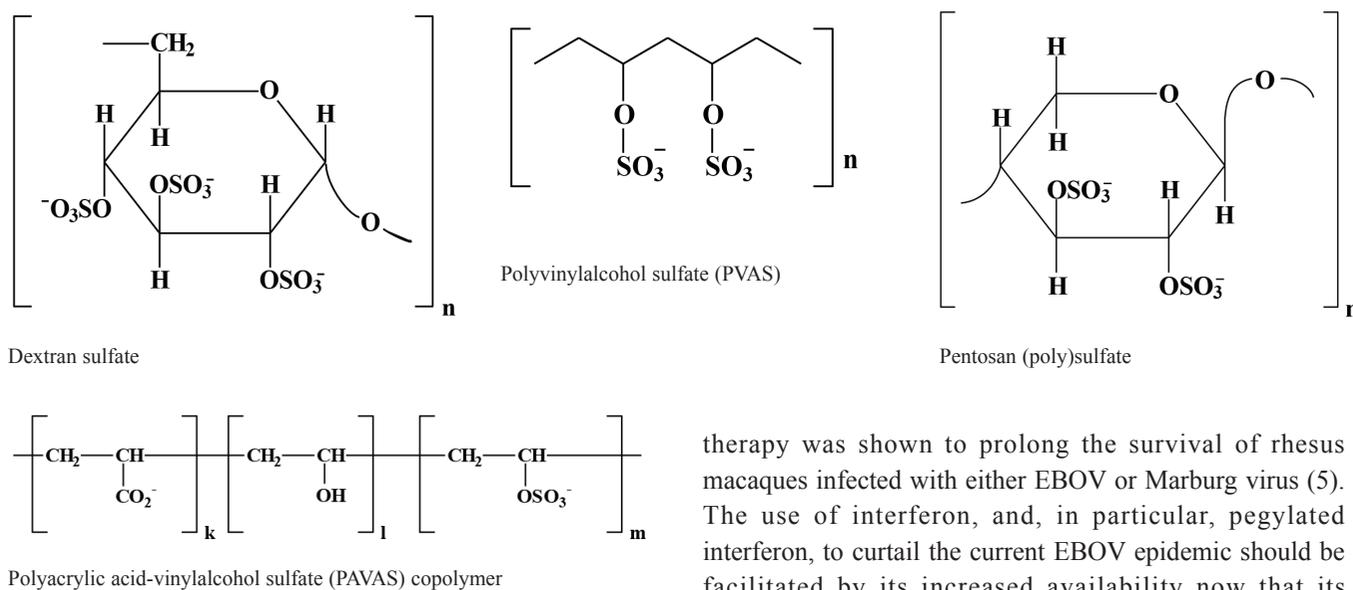


Fig. 1. Sulfated polysaccharides.

to the *rhabdoviridae* (with rabies virus as the prototype). In the 1960s, when I started my career with Prof. Piet De Somer as my mentor, Ebola and Marburg had not yet been discovered: Ebola virus (EBOV) was first isolated by Pattyn et al. in 1976 (2), that is 5 years before AIDS would be identified as a well-defined disease (the viral origin of this disease would be identified two years later, in 1983). In the late 1960's, Maurice Hilleman's group at Merck found that interferon could be induced by double-stranded RNAs [i.e. poly(I).poly(C)]; Tom Merigan at Stanford found that interferon could be induced by a synthetic polyanion (pyran copolymer) and, in De Somer's Laboratory I found another synthetic polyanion (polyacrylic acid) as inducer of interferon. In 1968, I published, with my mentor as co-author, that both interferon and polyacrylic acid could protect newborn mice against a lethal VSV infection (3). Little I knew that almost 50 years later this observation could serve as a paradigm for an epidemic, Ebola, that would lead to a death toll of more than 10,000 victims in West Africa, and spread fear and concern over the whole world.

INTERFERON

Whenever a new virus infection emerges, or re-emerges, so does the interest in using interferon to combat this infection. This was the case in 2003 when SARS (severe acute respiratory syndrome) emerged (4), and it happened again with the current EBOV outbreak (5). In fact, interferon- β

therapy was shown to prolong the survival of rhesus macaques infected with either EBOV or Marburg virus (5). The use of interferon, and, in particular, pegylated interferon, to curtail the current EBOV epidemic should be facilitated by its increased availability now that its usefulness in the treatment of hepatitis C virus infections is dwindling down because of the growing impact of direct-acting antivirals (DAAs) to treat HCV infections. That interferon may be effective in the treatment of EBOV infections could somehow be presaged by the protective effects noted, now almost 50 years ago, by interferon and its inducers (i.e. polyacrylic acid) against VSV infection in newborn mice (3).

SULFATED POLYSACCHARIDES (Fig. 1)

Sulfated polysaccharides have been identified as potent and selective inhibitors of various enveloped viruses, in particular HIV, but also VSV (6). The prototype of this family is dextran sulfate, but mannan sulfate has proven almost 10-fold more potent against VSV in this respect (7). Pentosan polysulfate was described by Baba et al. (8) as a potent and selective HIV inhibitor. Dextran sulfate was first shown to be inhibitory to the replication of HIV in 1987 by Ueno and Kuno (9) and Ito et al. (10). Still in 1988, Baba and his coworkers (6) confirmed that the antiviral activity of sulfated polysaccharides included VSV, and that, as specifically shown for dextran sulfate, its inhibitory effect on HIV was due to the inhibition of virus binding (adsorption) to the cells (11), an observation that had been independently made by Mitsuya et al. (12) as well. Schols et al. (13) further described sulfated polymers such as PVAS (polyvinylalcohol sulfate) and its copolymer with polyacrylic acid (PAVAS) as potent and selective inhibitors of various enveloped viruses such as HSV, CMV, VSV, RSV, and toga-, arena- and retroviruses (including HIV). For dextran sulfate, inhibition of VSV replication was shown within a molecular weight range of 5,000 to 500,000

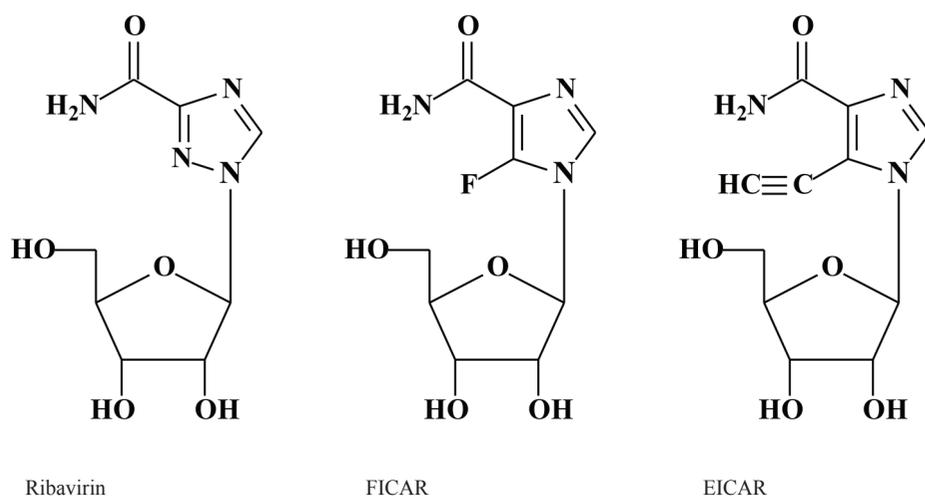


Fig. 2. IMP dehydrogenase inhibitors.

(14); curiously, heparin, while active against HIV, did not prove inhibitory to VSV (14). Antiviral activity against HIV and other enveloped viruses, including VSV, was later shown with a variety of sulfated polysaccharides extracted from seaweeds (15,16). It is obvious that all sulfated polymers, irrespective of their origin (synthetic or biologic), because of their activity against VSV, would deserve to be further evaluated for their activity against filoviruses such as EBOV. This advice may also be extended to the polyanionic (i.e. polysulfonate) dendrimers, which were found inhibitory to HIV but not evaluated against VSV (17).

IMP DEHYDROGENASE INHIBITORS (Fig. 2)

It is not evident that the IMP dehydrogenase (which converts IMP to XMP, that is then converted to GMP and thus replenishes the intracellular GTP pools) is an appropriate target for potential anti-VZV and/or –EBOV agents. Ribavirin [which has been identified in 1972 as a broad-spectrum antiviral agent (18,19)] is targeted at the IMP dehydrogenase (20), but it has only modest activity against VSV (21) and little or no activity against EBOV (22). Introduction of a fluorine in the imidazole moiety of the heterocyclic ring, thus resulting in the formation of FICAR (5-fluoro-1-β-D-ribofuranosylimidazole-4-carboxamide) decreases the anti-VSV potency of ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-4-carboxamide) (21), but introduction of a 5-ethynyl function as in EICAR (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide) markedly increases the anti-VSV potency (curiously, EICAR was found to inhibit VSV replication in HeLa cells at 4 μg/ml, while it was inactive against VSV in primary rabbit kidney (PRK) cells (23). Being an IMP dehydrogenase inhibitor, EICAR, like ribavirin, may not be primarily

directed to the treatment of virus infections (HCV is an apparent exception, but here ribavirin is mainly acting as an immunosuppressive rather than antiviral agent). EICAR was originally envisaged as an anti-leukemic agent (24). Its potent inhibitory effect on IMP dehydrogenase (25) may point to its role as an immunosuppressive agent.

CYCLOPENTYL CYTOSINE (CARBODINE) AND CYCLOPENTENYL CYTOSINE (Fig. 3)

At a certain time, about 25 years ago, carbodine (carbocyclic cytidine, C-Cyd) (26) and cyclopentenyl cytosine, Ce-Cyd (27) generated much interest as broad-spectrum antiviral agents. With an IC_{50} of 0.7 μg/ml (Ce-Cyd) and 4 μg/ml (C-Cyd) against VSV (in PRK cells), this activity also extended to the family of the rhabdoviruses, which, again, could herald activity against the filovirus EBOV. Moreover,

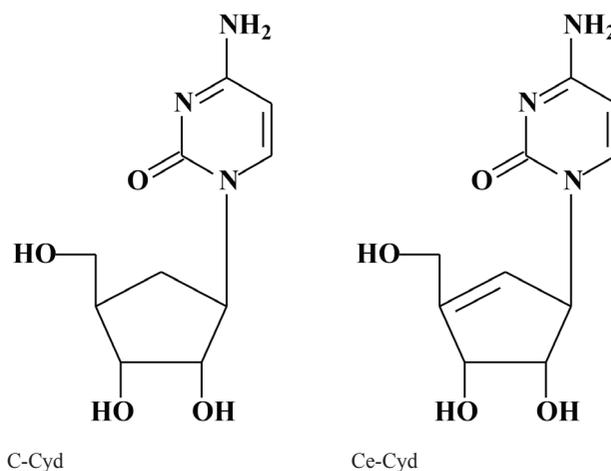


Fig. 3. Cyclopentyl and cyclopentenyl cytosine.

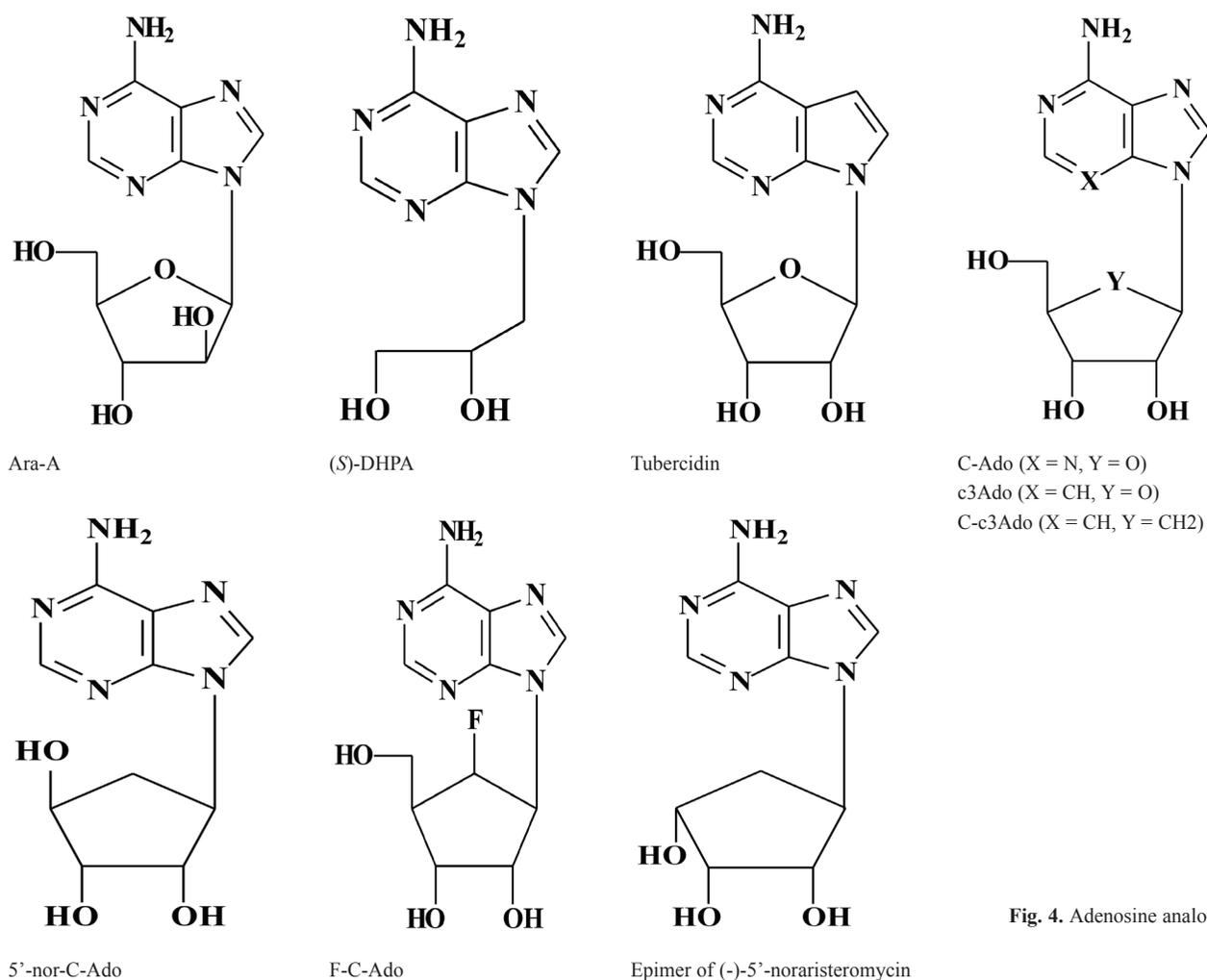


Fig. 4. Adenosine analogues.

C-Cyd and Ce-Cyd are targeted at the CTP synthetase, which converts UTP to CTP, and thus plays an important role in *de novo* biosynthesis of pyrimidine mononucleotides. This role could be considered as additive to the antiviral or antimetabolic action of pyrazofurin which is targeted at OMP decarboxylase that converts OMP to UMP, and thus interferes with a step higher up in *de novo* biosynthesis of pyrimidine mononucleotides. Pyrazofurin has been found to be extremely potent in inhibiting VSV replication.

SAH HYDROLASE: ADENOSINE ANALOGUES (Fig. 4)

Vidarabine (ara-A) has been known for half of a century as an antiviral agent specifically active against DNA viruses such as herpes simplex virus and vaccinia virus (28,29). The fact that it exhibited some activity against a (-)RNA virus, VSV (30) was therefore considered as a *curiosum*, and so was the anti-VSV activity of (*S*)-9-(2,3-

dihydroxypropyl)adenine [(*S*)-DHPA] (31). However, the discovery of 3-deazaadenosine (c^3 Ado), the carbocyclic analogue of adenosine (C-Ado) and 3-deazaadenosine (C- c^3 Ado), which had all three been recognized as S-adenosylhomocysteine (SAH) hydrolase inhibitors, confirmed that all these compounds acted against VSV by inhibiting the SAH hydrolase (32), and, in fact, a close correlation was found between the anti-VSV activity and SAH hydrolase inhibition (33). Tubercidin (7-deazaadenosine, c^7 Ado) was not included in this comparative study: it proved extremely potent (minimal inhibitory concentration (MIC): 0.0007 μ g/ml) in its anti-VSV activity, but also quite cytotoxic (0.4 μ g/ml) (34,35). Tubercidin and its related analogues toyocamycin and sangivamycin must have various effects other than SAH hydrolase inhibition pertaining to their cytotoxicity. In addition to tubercidin, toyocamycin and sangivamycin, C-nucleoside analogue, formycin, may seem too toxic to be further explored from an antiviral viewpoint (36,37).

SAH HYDROLASE: NEPLANOCIN ANALOGUES (Fig. 5)

With the discovery of neplanocin A or (-)-9-[(*trans*-2,*trans*-3-dihydroxy-4-(hydroxymethyl)cyclopent-4-enyl]adenine, 3-deazaneplanocin A and their 5'-nor derivatives, 9-(*trans*-2',*trans*-3'-dihydroxycyclopent-4'-enyl)adenine (DHCeA) and 9-(*trans*-2',*trans*-3'-dihydroxycyclopent-4'-enyl)-3-deazadenine (c³DHCeA) (38,39), the link between SAH hydrolase inhibition and antiviral activity, especially against VSV, was clearly corroborated, and again, a close correlation was found between inhibition of SAH hydrolase and anti-VSV activity (40,41). In the latter article, I postulated that the viruses proven particularly sensitive to inhibition by SAH hydrolase inhibitors were the poxviridae, paramyxoviridae, rhabdoviridae (including rabies, infectious hematopoietic necrosis virus, and VSV) and reoviridae. Ten years later, Bray would demonstrate that

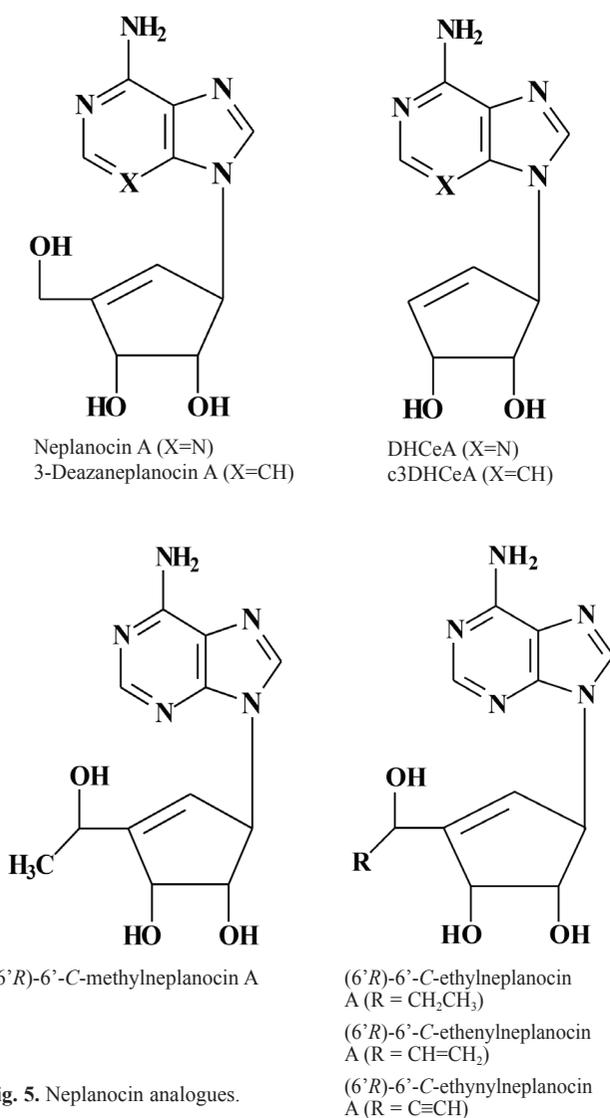


Fig. 5. Neplanocin analogues.

3-deazaneplanocin A was exquisitely active, *in vivo*, against EBOV (42,43). In addition to neplanocin A and 3-deazaneplanocin A (MIC₅₀: 0.07 μg/ml for VSV), various other aristeromycin and neplanocin A analogues are active against VSV at an MIC of *circa* 0.1 μg/ml: (±)5'-noraristeromycin (44), (-)5'-noraristeromycin (45,46), and 3-deaza-5'-noraristeromycin (46); (6'*R*)-6'-C-methylnepanocin A (47), (6'*R*)-6'-C-ethenyl (or ethynyl) neplanocin A (48); (±)-6'-β-D-fluoroaristeromycin (F-C-Ado) (49); and *epi*(-)-5'-noraristeromycin (50). All these compounds may be assumed to act as SAH hydrolase inhibitors (51) and should be further explored for their potential as anti-EBOV drug candidates.

FAVIPIRAVIR (T-705) (Fig. 6)

Favipiravir is the only pyrazine compound shown to be antivirally active (52,53). It is active against both (-)RNA

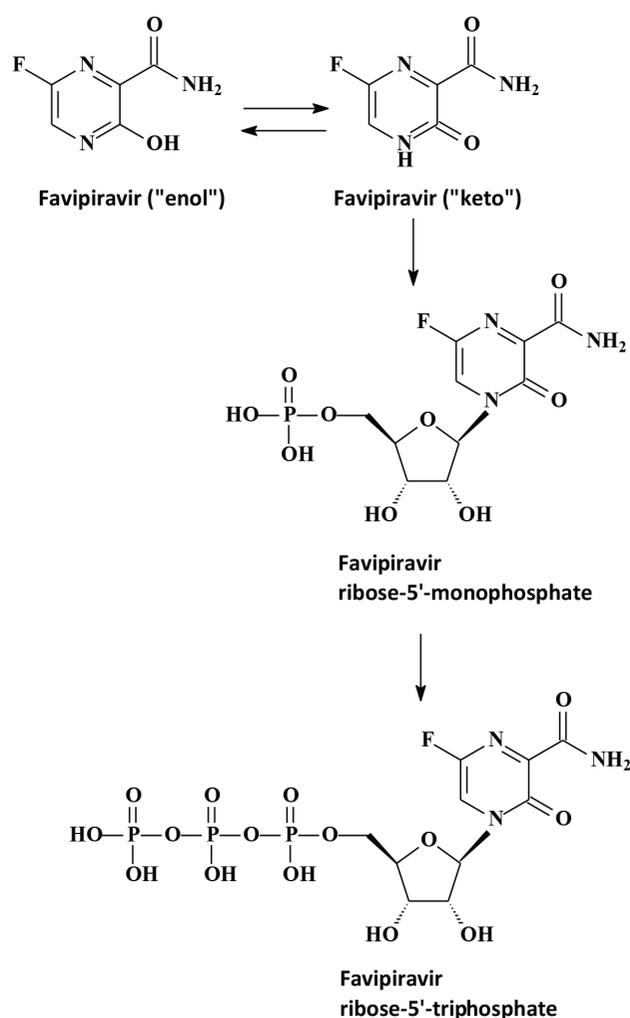


Fig. 6. Favipiravir and its metabolites.

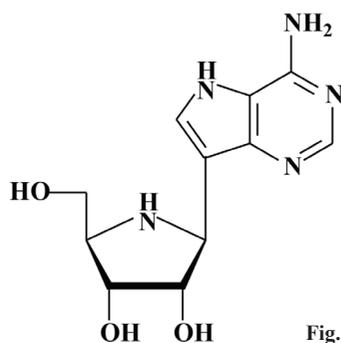


Fig. 7. BCX4430.

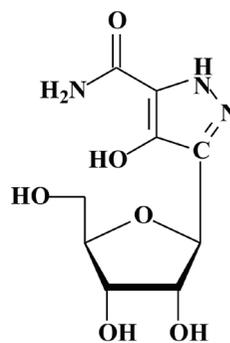


Fig. 8. Pyrazofurin.

viruses (i.e. orthomyxo-, paramyxo-, arena-, bunya-, hantaviruses) and (+)RNA viruses (flavi-, picorna- and noroviruses). Although its potential activity against rhabdoviridae (rabies, VSV) was not assessed, it was shown to be efficacious *in vivo*, in mice, against EBOV infection (54,55). The compound (trade name: Avigan®) has been approved in Japan for the treatment of influenza A virus infections, and be made available in (sufficiently ?) large quantities for the treatment of EBOV infection in West Africa. Favipiravir is assumed to be targeted at the viral RNA polymerase. To this end, the compound should be converted by a phosphoribosyl transferase (similar to orotic acid, adenine and hypoxanthine-guanine) to its ribosylmonophosphate, and then converted to its triphosphate, before interacting at the viral RNA polymerase, presumably in direct competition with GTP.

BCX4430 (Fig. 7)

As mentioned by Warren et al. (56), BCX4430, a C-nucleoside, was synthesized as part of a small-molecule library designed as inhibitors of viral RNA polymerase activity. BCX4430 would inhibit viral RNA polymerase through a non-obligate RNA chain termination, obviously after its (intracellular) phosphorylation to BCX4430 triphosphate. BCX4430 proved particularly active against picorna-, flavi-, orthomyxo-, paramyxo- and filoviruses (its activity against rhabdoviruses such as VSV was, unfortunately, not determined). *In vivo*, BCX4430 completely protected cynomolgus macaques against Marburg virus infection, and claimed to be the first compound shown to protect non-human primates from a filovirus infection (56). This may herald potential efficacy in the treatment of EBOV infection in humans. BCX4430 has also been shown to offer complete protection from mortality in hamsters infected with yellow fever virus (57).

Pyrazofurin (Fig. 8)

Pyrazofurin, a C-nucleoside, was found to be extremely

potent against VSV, irrespective of the cell culture used: primary rabbit kidney (PRK) (MIC: 0.01 µg/ml), human skin fibroblast (HSF) (MIC: 0.04 µg/ml) and HeLa (MIC: 0.02 µg/ml) (58). Pyrazofurin was about 1,000-fold more potent against VSV than ribavirin. However, its efficacy against VSV *in vivo* could not be assessed as the compound proved too toxic to mice: its 50% lethal dose (LD₅₀) was approximately 5 mg/kg per day. This *in vivo* toxicity also hampered the further evaluation of pyrazofurin against murine leukemia virus, which, like VSV, appeared exquisitely sensitive (MIC: 0.01 µg/ml) to inhibition by pyrazofurin (59). Pyrazofurin (originally named pyrazomycin) has since long been shown to inhibit *de novo* pyrimidine mononucleotide biosynthesis at the level of orotidylic acid (OMP) decarboxylase (which converts OMP to UMP) (60,61).

EBOV INHIBITORS INTERACTING WITH VIRAL ENTRY (Fig. 9)

Mannose-specific lectins

Griffithsin and similar lectins that bind to the terminal mannose residues of the glycoproteins may be potentially useful in the treatment of EBOV infections (62,63).

Endoplasmic reticulum (ER) glucosidase inhibitors

The imino sugar 1-deoxynojirimycin is a glucose mimic, with a nitrogen atom replacing the oxygen, that inhibits the ER α-glucosidases I and II, which are essential in the maturation of viral envelope glycoproteins (64). Its derivatives IHVR11029, IHVR17028 and IHVR19029 were shown to protect mice against the mortality of Marburg and EBOV infections (65).

Benzylpiperazine adamantane diamides

EBOV entry into the host cells requires the cholesterol transporter Niemann-Pick C1 (66), and this process can be blocked by benzylpiperazine adamantane diamides (67).

Rhodamine derivatives

The rhodamine derivative LJ-001 inhibits the cell entry of

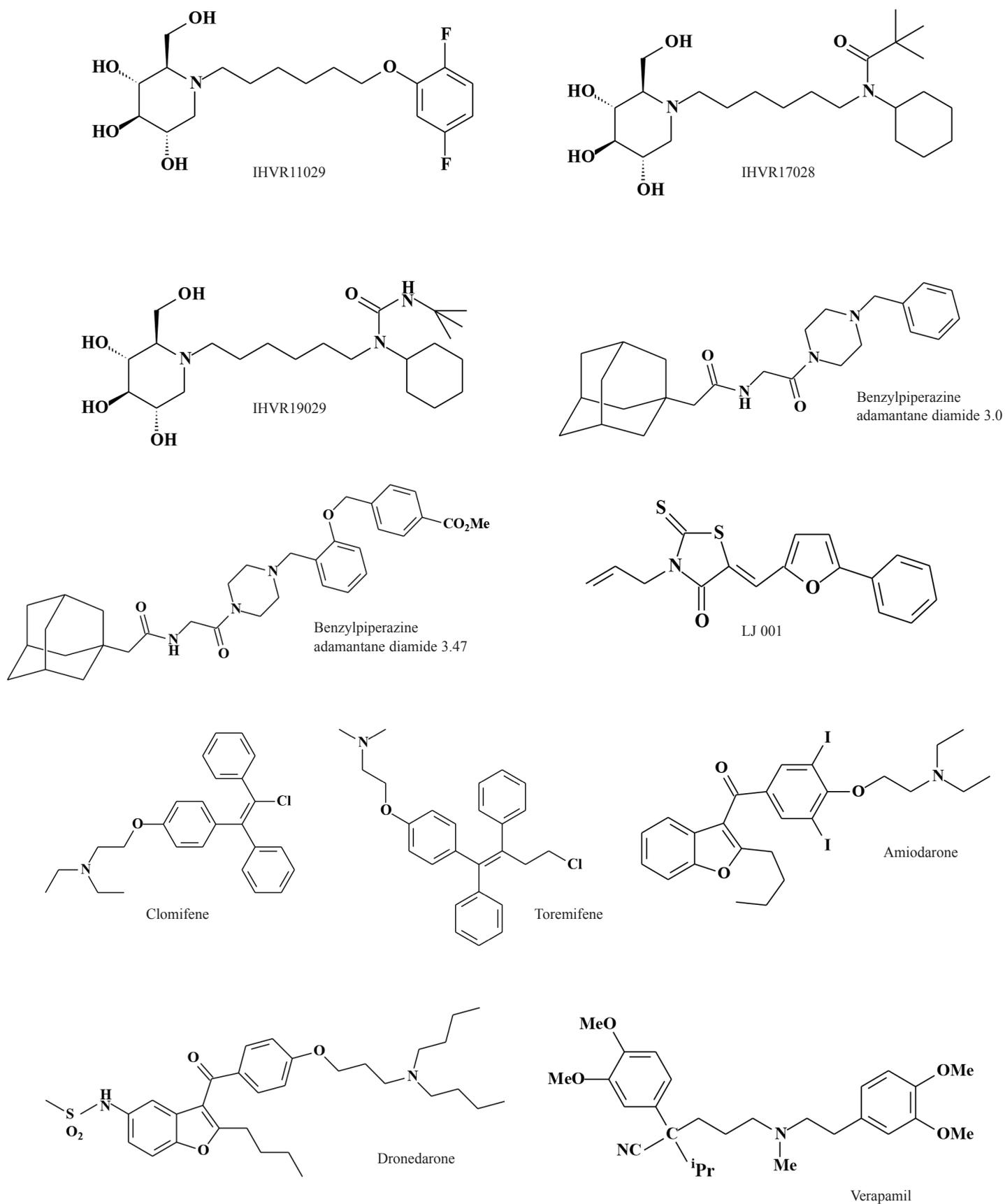


Fig. 9. Miscellaneous compounds inhibiting EBOV entry.

various enveloped viruses such as influenza A, HIV, pox-, arena-, bunya-, paramyxo-, flavi- and filoviruses, including EBOV (68).

Selective estrogen receptor modulators (SERMS)

SERMS (i.e. clomifene and toremifene), through an off-target, interfere with a late step of EBOV entry, thereby preventing the fusion process (69).

Ion channel blockers

The ion channel blockers amiodarone, dronedarone and verapamil were found to inhibit the cell entry of filoviruses (i.e. EBOV) (70) at the concentrations required for anti-arrhythmic therapy in humans (i.e. 1.5-2.5 µg/ml).

CHLOROQUINE (Fig. 10)

Chloroquine has been known since 1934 as an anti-malaria agent. Concomitantly with, and subsequently to, the emergence of HIV, SARS coronavirus and finally EBOV, chloroquine was shown to inhibit HIV (71), SARS coronavirus (72), and EBOV (73). It inhibits both the endocytosis and exocytosis of virus particles, and in addition, downregulates IFN- γ and TNF- α production (74).

CONCLUSIONS

Furtherst advanced in the treatment of EBOV infections is favipiravir (T-705), also because its human use has proved safe and efficacious in the treatment of influenza virus infections. It is targeted at the viral RNA polymerase; although effective against (+)RNA and (-)RNA viruses, its activity against the (-)RNA rhabdoviruses (i.e. VSV) has not been assessed. The latter is true for BCX4430 as well, which in addition is a C-nucleoside, for which the safety/toxicity profile in humans remains to be ascertained. Plenty of S-adenosylhomocysteine hydrolase (SAH) inhibitors have been shown to be highly active against VSV, and one of them, 3-deazaneplanocin A, has also been shown to be effective against EBOV. Pyrazofurin is an highly potent

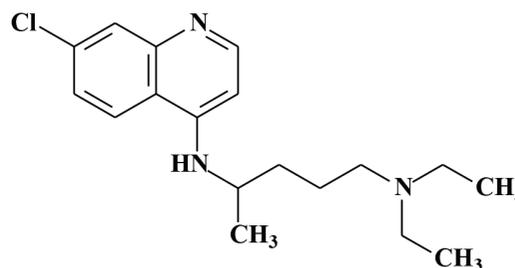


Fig. 10. Chloroquine (Nivaquine).

inhibitor of VSV, but its activity against EBOV has not been evaluated. Like BCX4430, pyrazofurin is a C-nucleoside, which may be considered as a liability for its therapeutic (antiviral) usefulness. Ribavirin and other IMP dehydrogenase inhibitors may not seem particularly effective against VSV and EBOV. C-Cyd and Ce-Cyd are sufficiently active against VSV to be further evaluated for their potential activity against EBOV. This suggestion should also be extended to the sulfated polysaccharides which have proved quite effective *in vitro* against VSV, never evaluated against EBOV, but, once upon a time, considered for their potential in the treatment of HIV infections. Being a prodrug of cidofovir, there is no rationale whatsoever for the activity of brincidofovir (CMX001) against EBOV. Given its historically founded activity against VSV, interferon should be entertained for the treatment of EBOV infections. In the meantime, numerous compounds licensed for the most disparate clinical indications have been found to interfere with the cell entry of EBOV. And the list of antiviral drugs to be envisaged for their potential use against EBOV should be incomplete if it were not finalized by chloroquine.

ACKNOWLEDGMENTS

I am most grateful to Mrs. Christiane Callebaut for her proficient editorial assistance.

EBOV enfeksiyonlarının tedavisi amacıyla ilaç geliştirme sürecinde VSV örneği

ÖZET

İnsanlarda görülen Ebola virüs (EBOV) enfeksiyonlarının tedavisinde kullanılmak üzere bir antiviral ilaç henüz sağlık otoritelerince onaylanmamıştır. Bu amaçla kullanılmak üzere onaylanmaya en yakın olan ilaç bir viral RNA polimeraz inhibitörü olan favipiravir (T-705)'tir. Üzerinde çalışılan diğer bileşikler ise bir viral RNA polimeraz inhibitörü olan BCX4430 ve S-adenosilhomosistein (SAH) hidrolaz inhibitörü olan 3-deazaneplanosin A'dır. Diğer endikasyonlar için onaylanan bileşiklerden Ebola virüs (EBOV) enfeksiyonlarının tedavisinde

kullanılmak üzere denenilenlerin ise Ebola virüs'ün hücre penetrasyon faktörleri ile etkileştiği gözlenmiştir. Pirazofurin gibi bazı bileşiklerin ise veziküler stomatitis virüs'ün (VSV) potansiyel inhibitörleri olduğu tespit edilmiştir. VSV, *rhabdoviridae* ailesine ait bir virüstür ve bu virüs ailesi EBOV ve Marburg virüs'ünün mensubu olduğu *filoviridae* virüs ailesi ile yüksek oranda benzerlik göstermektedir. EBOV ve Marburg virüs'ü biyogüvenlik derecesi 4 oranında çalışma güvenliği gerektirirken VSV ile mutata güvenlik koşullarında çalışılabilmektedir.

Anahtar Kelimeler: Ebola virüs (EBOV); veziküler stomatitis virüs (VSV); *rhabdoviridae*; *filoviridae*; favipiravir; BCX4430; pirazofurin; SAH hidrolaz

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