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Commentary

# Combating enterovirus replication: State-of-the-art on antiviral research

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ABSTRACT

Enteroviruses form an important genus within the large family of Picornaviridae. They are small, non-enveloped (+)RNA viruses, many of which are important pathogens in human and veterinary science. Despite their huge medical and socio-economical impact, there is still no approved antiviral therapy at hand for the treatment of these infections. Three capsid-targeting molecules (pleconaril, BTA-798 and V-073) are in clinical development. Pleconaril and BTA-798 are in phase II clinical trials for the treatment of enterovirus-induced sepsis syndrome and rhinovirus-induced aggravation of pre-existing asthma or COPD respectively. V-073 is in preclinical development for the treatment of poliovirus infections in the context of the worldwide polio eradication program. The capsid binding molecules have shown good in vitro potency against a number of enterovirus species, but lack activity against others. Another potential drawback of capsid inhibitors in the clinical setting could be the rapid emergence of drug resistance. It will therefore be important to develop inhibitors that affect other stages in the viral replication cycle. Several viral proteins, such as the viral 3C protease, the putative 2C helicase and the 3D RNA-dependent RNA polymerase may be/are excellent targets for inhibition of viral replication. Also host cell factors that are crucial in viral replication may be considered as potential targets for an antiviral approach. Unraveling these complex virus–host interactions will also provide better insights into the replication of enteroviruses. This review aims to summarize and discuss known inhibitors and potential viral and cellular targets for antiviral therapy against enteroviruses.



## 1. Introduction

Enteroviruses form a large genus containing many different pathogens that can cause a variety of diseases in man. Within the Picornavirus family, hepatitis A virus (genus hepatovirus) and poliovirus (genus enterovirus) are the only human pathogens for which an efficient vaccine is at hand. Efforts to develop enterovirus inhibitors remained confined to the human rhinoviruses, the major causative agents of the common cold. This search for rhinovirus inhibitors experienced a boost in the 1980s with the determination of the crystal structure of HRV14 [1]. Given the fact that the viral capsid indeed proved to be a promising antiviral target, the field of antiviral drug discovery for enteroviruses remained largely and for many years restricted to this particular target. Despite several successes, it proved challenging to obtain market authorization for a drug to treat the common cold, one of the reasons being that such drug should be extremely safe. Hence, the efforts undertaken at that time remained unfruitful. Today, there is strong interest again in the development of anti-rhinovirus drugs, as well as an insistent need for an efficient treatment of enteroviral infections, such as those caused by enterovirus 71. Drugs against poliovirus to aid in the polio endgame are urgently required as well.

Recent data provide strong evidence that rhinoviruses are implicated in exacerbations of asthma and chronic obstructive pulmonary disease (COPD) [2,3]. COPD is predicted by the World Health Organization (WHO) to become the third leading cause of death worldwide by the year 2030 [89].

In 1988, the "Global Polio Eradication Initiative" (GPEI) was launched and the idea was to eradicate polio worldwide by the year 2000 through large scale vaccinations with the live, attenuated oral polio vaccine (OPV) [4]. Today, more than 10 years past the initial deadline, the virus is still endemic in several countries and regions that had been claimed "polio-free" report new epidemics from time to time [90]. Once transmission of wild poliovirus (PV) has officially been interrupted, the WHO will initiate a plan to discontinue the use of OPV, in order to minimize the risk for vaccine-associated paralytic poliomyelitis (VAPP), chronic infection of immunodeficient patients or reestablishment of PV in the environment through circulating vaccine-derived polioviruses [5]. The potential role of an antiviral in the context of polio eradication was addressed in 2005 by a panel of experts convened by the National Research Council. It was concluded that

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it would be appropriate, and possibly essential, to develop at least two anti-poliovirus drugs, preferably with a different mode of action, to be used in the post-OPV era [6].

Enterovirus 71 is increasingly reported to cause serious epidemics, in particular among children and on the Asian continent [7]. At certain occasions, enterovirus 71 can lead to severe and even fatal neurological complications such as brain stem encephalitis. Treatment options are still limited to supportive and symptomatic care, as specific antivirals are not at hand. Apart from enterovirus 71, many other enteroviruses have been reported to cause severe diseases, including viral myocarditis, neonatal sepsis and fulminant pancreatitis [8].

So far no drugs have been approved and just a few are under clinical development.

## 2. Small molecule inhibitors of enterovirus replication

### 2.1. Inhibitors of attachment, entry and/or uncoating

As mentioned above, the viral capsid was one of the earliest viral proteins that was identified as a potential target for inhibition of viral replication (Fig. 1). In fact, one of the earliest discovered classes of enterovirus inhibitors belong to a series of compounds that are currently known as "WIN" compounds, referring to Sterling Winthrop, where they were developed [9]. With the report of the atomic resolution structure of human rhinovirus 14 [1], it was recognized that a large cleft ("canyon") is present on each icosahedral face of the virus. Structural studies of several WIN compounds complexed with human rhinovirus 14 revealed that these compounds bind into the hydrophobic pocket, underneath the canyon floor [10]. This binding event induces conformational changes, thereby increasing the rigidity of the virion and at the same time decreasing the ability of the virion to interact with its receptor [11] (Fig. 2).

The WIN compounds were accidentally discovered in a synthesis project for juvenile hormone mimetics [9]. Despite their moderate antiviral activity at the initial stages of the project, the compounds underwent several stepwise modifications to improve their antiviral properties. With the development of WIN63843 (pleconaril) (Fig. 3A), a compound was obtained with favourable drug profile [12] and broad spectrum anti-enterovirus activity [13]. In 1996, Viropharma made the drug available for compassionate use in patients with potentially life-threatening enterovirus infections. Outcomes of clinical trials with pleconaril revealed promising [14,15], as well as rather negative outcomes [16]. In 2002, following submission of a new drug application to use pleconaril for the treatment of the common cold, the drug was rejected by the FDA mainly due to safety issues [17]. Pleconaril has

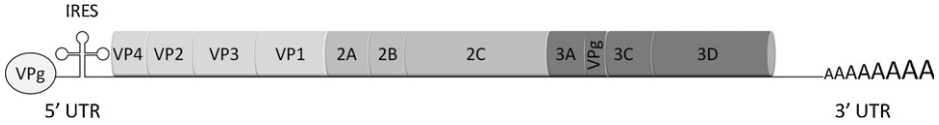


**Fig. 1.** Organization of the enterovirus genome. The genome consists of a single-stranded, positive sense RNA of approximately 7.5 kb in length. At the 5′-end, a small protein called VPg is covalently linked to the viral genome and is involved in the initiation of viral RNA replication. This VPg is followed by the 5′ untranslated region (UTR), the protein coding region, the 3′ UTR and a poly(A) tail. Coding regions for the viral proteins are indicated. The genome is divided into a structural region (consisting of viral protein (VP 1–4)) and a non-structural region comprising two proteases (2A and 3C), one polymerase (3D), one ATPase (2C) and four other proteins that, either cleaved or as a precursor, are involved in viral replication.

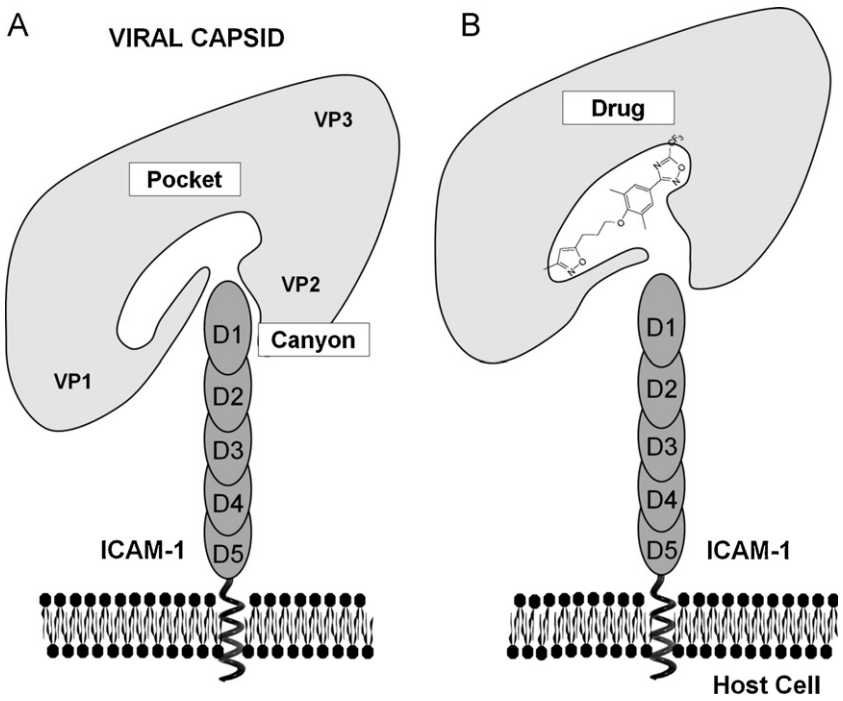


**Fig. 2.** Mechanism of action of capsid binders. Schematic representation of the interaction between ICAM-1 and major group rhinoviruses. Left (A): ICAM-1 binds into the canyon, surrounding each fivefold axis, inducing conformational changes that eventually lead to uncoating of the virus and release of the viral RNA. Right (B): Binding of a "capsid binder" into the hydrophobic pocket, underneath the canyon floor. This binding event induces conformational changes, thereby (i) increasing the rigidity of the virion (preventing uncoating and subsequent release of viral RNA) and at the same time (ii) decreasing the ability of the virion to interact with its receptor.

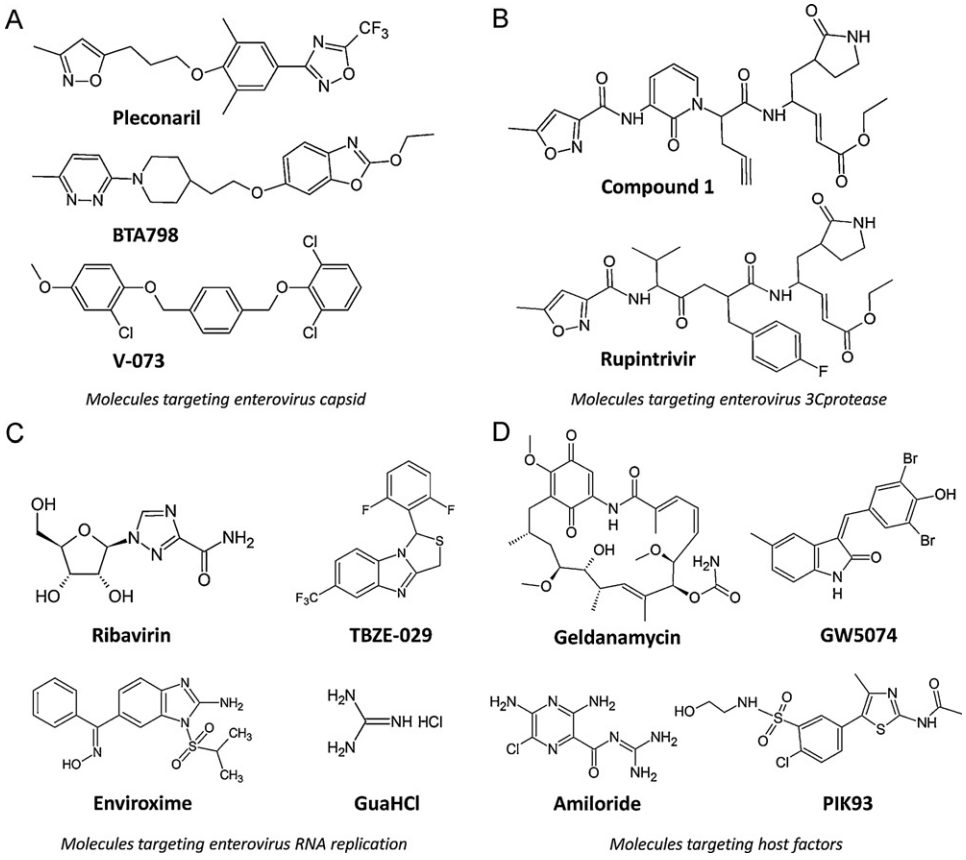


**Fig. 3.** Structural formulae of molecules targeting enterovirus replication.

been licensed to Schering-Plough in 2003 where the molecule has been under development as a nasal spray for the treatment of common cold symptoms and HRV-induced exacerbations of asthma/COPD in high-risk patients. A phase II rhinovirus challenging study has been completed in 2007, but results of this study have not (yet) been disclosed [91]. At present, pleconaril is being investigated in a phase II study for the treatment of enteroviral sepsis syndrome in neonates [92] (Table 1).

Around the same time as pleconaril, pirodavir was developed at Janssen Pharmaceutica [18]. Akin to pleconaril, this molecule with broad-spectrum anti-rhinovirus activity was demonstrated to act as a capsid binding compound [19]. At Biota holdings, structural analogues of pirodavir were synthesized to overcome the chemical instability of the compound [20]. The most potent congener in this series was BTA-798 (Fig. 3A) which proved in vitro 10-fold more active than pleconaril and has a longer half life and good oral bioavailability [21]. BTA-798 is currently being developed at Biota for the treatment of rhinovirus infections in high-risk patients, including patients with asthma, cystic fibrosis, COPD and transplant patients. Recently, a phase IIa double-blind challenge study with HRV was successful in demonstrating proof-of-concept in humans and was shown to reduce the incidence and severity of HRV infection (Table 1).

In light of the polio eradication programme, V-073 (Fig. 3A) is being investigated at ViroDefense. This compound, previously designated SCH 48973, was shown to exhibit potent and broad spectrum activity against a range of poliovirus clinical isolates [22]. The compound binds at the innermost end of the hydrophobic pocket within VP1 [23], but it does not prevent attachment of the virus to the host cell. Instead, it exerts its antiviral activity after the initial stage of viral uncoating [24] (Table 1).

The fact that the structure of the viral capsid is created the opportunity to rationally design compounds that perfectly fit within the hydrophobic pocket with high affinity. As discussed below, for many non-structural proteins the crystal structure still remains to be determined and this knowledge gap often poses an impediment in the development of more potent and selective inhibitors.

Most if not all of the capsid binding agents described above, readily select for drug-resistance [25]. This is due to the error-prone nature of the viral polymerase and the fact that the viral structural proteins are less well conserved than several of the non-structural proteins. Thus, to circumvent the emergence of drug-resistance it will be important to have drugs at hand that have a non-overlapping resistance profile with the capsid binding agents, possibly a combination of two molecules with a different molecular target [26].

### 2.2. Inhibitors that target proteolytic enzymes

The viral polyprotein is co-translationally processed in a series of primary and secondary proteolytic cleavages by virus-encoded proteases $2A^{pro}$, $3C^{pro}/3CD^{pro}$ generating several precursor and mature proteins [27] (Fig. 1). Proteinases can thus be considered as essential for viral replication. In addition, enterovirus-encoded proteinases also cleave several host cell factors involved in transcription [28], nucleo-cytoplasmic transport [29], cap-dependent translation [30,31] and alteration of stress granules and processing bodies [32]. Given the fact that proteinases, especially $3C^{pro}$ (which is a trypsin-like, cysteine protease with a preference of cleaving $P_1$-Gln-$P_{1'}$-Gly bonds), are indispensable for viral maturation, the strict conservation of the catalytic residues among

**Table 1**
Overview of clinical trials with anti-enterovirus drugs.

| Drug | Pharmaceutical company | Mode of action | Application | Status[a] | Remarks | References |
|---|---|---|---|---|---|---|
| Pleconaril | Sterling Winthrop/ Viropharma | Capsid binder | Compassionate use for life-threatening enterovirus infections | Clinical development halted | Inconsistent results | [14–16] |
| Pleconaril | Sterling Winthrop/ Viropharma | Capsid binder | Common cold symptoms in HRV infection | New drug application rejected by FDA in 2002 | Safety profile of the drug disputed | [17] |
| Pleconaril | Schering-Plough (2003) | Capsid binder | HRV infections in high-risk patients, common cold symptoms | Phase II challenge study completed | No results disclosed so far | [91] |
| Pleconaril | Schering-Plough (2003) | Capsid binder | Enteroviral sepsis syndrome in neonates | Phase II challenge study ongoing | | [92] |
| BTA-798 | Biota Holdings Ltd | Capsid binder | HRV infections in high-risk patients | Phase IIa double-blind challenge study completed | Successful proof of concept in humans | www.biota.com.au |
| V-073 | ViroDefense Inc | Capsid binder | Management of post-polio eradication | Lead for preclinical development | | M. Collett; personal communication |
| Rupintrivir | Agouron/Pfizer Inc | Protease 3C inhibitor | Common cold symptoms in HRV infection | Discontinued at Phase II | Insufficient therapeutic effect in natural infection studies | [44] |
| Compound 1 | Agouron/Pfizer Inc | Protease 3C inhibitor | Common cold symptoms in HRV infection | Discontinued at Phase I | No further clinical development is planned | [46] |
| Enviroxime | Lilly Research Laboratories | Unknown; targets 3A (and possibly other viral/cellular proteins) | Common cold symptoms in HRV infection | Discontinued at Phase II | Insufficient therapeutic effect Gastro-intestinal side effects | [70] |

[a] Status at the time of publication.

enteroviruses [33] and the fact that viral proteases share little sequence similarities with cellular proteases [34], makes these proteins very attractive targets for the development of more and better chemotherapeutic agents.

Inhibitors of proteolytic enzymes can be classified into two structurally distinct groups: peptidic inhibitors, based on the cleavage specificity of the enzyme, and nonpeptidic inhibitors. Proteolytic enzymes can be inhibited via two known mechanisms: reversible tight-binding reactions and irreversible trapping reactions. Inhibition via this latter mechanism will irreversibly "trap" the enzyme upon binding by triggering a conformational change involving key amino acids required for enzymatic activity. In reversible tight-binding reactions, the inhibitor will interact with the active site of the enzyme and will compete with the natural peptide substrate [35,36]. A variety of protease inhibitors have been discovered and developed over the years. The first inhibitors were competitive reversible peptidomimetics [37,38]. With the availability of computational technologies, crystal structures and in silico modeling [39] other, more specific Michael-acceptor containing peptidic inhibitors, were developed that were able to trap the protease irreversibly by forming a stable covalent complex. From these inhibitors, several structurally related analogues were synthesized of which rupintrivir (Fig. 3B) inhibited $3C^{pro}$ most potently [39]. This molecule proved to be very active against a broad panel of rhino- and enteroviruses and was selected for clinical trials [33,40]. Due to poor oral bioavailability, rupintrivir was administered intranasally [41] and was found to be well tolerated and reduced symptoms and viral load in HRV challenge trials [42,43]. However, in naturally infected patients no reduction in disease severity and viral load was noted and the compound was therefore halted from further development [44] (Table 1). Because of the poor bioavailability of this peptidomimetic compound, a nonpeptidic, orally bioavailable analogue was designed (Fig. 3B) [44,45]. Compound 1, as the molecule was designated, exhibited potent and broad spectrum anti-rhinovirus activity, and was well tolerated in phase I clinical studies. However, clinical development of this compound was not persued either [46] (Table 1).

The crystal structure of several picornavirus proteases (with or without cleavage substrate) has been solved and should facilitate the rational design and the development of novel protease-targeting inhibitors [47,48]. Proteases as antiviral targets have already proven their value in the treatment of HIV- and HCV-infected patients [36].

### 2.3. Inhibitors that target the RNA replication machinery

Upon receptor binding and entry into the cytoplasm of the host cell, viral RNA will serve as a template for protein translation and RNA replication. RNA replication occurs within membranous replication complexes and requires the participation of many host cell factors, viral precursors and mature proteins, including 2B, 2C, 2BC, 3A, 3B, 3AB, 3C, 3D and 3CD [49] (Fig. 1). Some of these proteins are already being studied intensively as potential targets for antiviral drug development. The most important findings will be summarized below.

#### 2.3.1. Targeting 2C protein

2C protein is highly conserved among picornaviruses [50] and involved in many critical functions, hence posing an excellent target for the development of broad spectrum antivirals. Despite the fact that this protein has been studied intensively, its complete role in viral RNA replication has not yet been elucidated. It has been documented that 2C is involved in the rearrangement of host cellular membranes [51], RNA binding [52], binding to various viral- and host proteins [53,54], morphogenesis of infectious virus

particles [53], ATPase- and GTPase activity [55] and even uncoating [56]. The ATPase domain of enterovirus protein 2C contains three conserved motifs (A, B and C) which are characteristic for the NTPases/helicases of superfamily III [57]. Despite the fact that the presence of these motifs suggests a helicase function for protein 2C, attempts to demonstrate such enzymatic activity remained unsuccessful. Of the several 2C-targeting compounds that have been reported so far, guanidine hydrochloride (GuaHCl) (Fig. 3C) is the most extensively studied. This compound inhibits poliovirus, several coxsackie- and echoviruses, foot-and-mouth disease virus (FMDV), but not hepatitis A virus (HAV) [58]. It was demonstrated that GuaHCl affects poliovirus replication at multiple levels, including blocking the initiation of negative, but not positive strand RNA synthesis, nor RNA chain elongation [59]. Furthermore, GuaHCl prevents binding of 2C or its precursor(s) to the host membranes [60] and finally, one study also demonstrated the inhibition of ATPase activity by GuaHCl [61]. Other 2C-targeting molecules, including 2-(α-hydroxybenzyl)-benzimidazole (HBB) [58] and a substituted benzimidazole, 1-(4-fluorophenyl)-2-[(4-imino-1,4-dihydropyridin-1-yl)methyl] (MRL-1237) [62] were reported to inhibit poliovirus and some coxsackieviruses. A substantial overlap between the resistance profiles of HBB and MRL-1237 has been reported, suggesting a similar mechanism of action [63]. The precise mode of action remains however to be elucidated. Finally, yet another benzimidazole derivative TBZE-029 (Fig. 3C) was reported to exhibit antiviral activity against several enteroviruses but not rhinoviruses or poliovirus. The compound does not inhibit 2C ATPase activity in vitro and may possibly target the enzymatic activity or the assembly of the putative ring helicase. TBZE-029-resistant variants also proved cross-resistant with other 2C-targeting molecules (GuaHCl, HBB and MRL-1237) [63]. In conclusion, 2C is one of the most conserved proteins among enteroviruses and is implicated in a multitude of critical stages in the viral replication cycle. Most 2C-targeting molecules select for mutations in the same region of the viral protein, which is apparently a 'hot spot' for accumulation of resistance mutations [63]. Thus far, the precise mechanism of action of this class of compounds remains unknown. Unraveling the precise mechanism of action may provide important insights in the biology of enteroviruses and may allow the rational design of more potent inhibitors.

*2.3.2. Targeting 3A protein*

Another indispensible, nonstructural protein is the multifunctional, small, hydrophobic protein 3A which is highly conserved among enteroviruses [64]. This protein is found in association with cellular membranes via a C-terminal hydrophobic region [65] and is implicated in many functions including RNA replication [65], host cell tropism and pathogenicity [66]. Moreover, it has been shown that 3A protein of enteroviruses B and C also affects ER-to-Golgi transport by inhibition of COP-I translocation to the secretory organelle membranes [64]. The ability of 3A protein to interfere with the host secretory pathway has been demonstrated to reduce the secretion of several cytokines and to downregulate TNF-receptors on the cell surface and MHC class I surface expression ([67] and references therein). Despite the fact that inhibition of ER-to-Golgi transport is not required for virus replication, it is thought that it may reflect a strategy by which pathogens might reduce the host cell's immune response and hence promote virulence during infection [68]. Recently, it has been reported how enterovirus protein 3A recruits phospatidylinositol-4-kinase IIIβ (PI4KIIIβ) to secretory organelle membranes. In doing so, a phosphatidylinositol-4-phosphate (PI4P) lipid-rich environment is created to which $3D^{pol}$ can bind and thus promote viral RNA replication [69]. As PI kinases appear to be essential host factors in the replication machinery, they may be considered as genuine targets for drug development. Thus far, only few molecules have been reported that target protein 3A of which enviroxime (Fig. 3C), a benzimidazole derivative, is the best studied [70] (Table 1). Several enterovirus inhibitors that select for mutations in 3A have been discovered, including TTP-8307 [67] and T-00127-HEV1 [71]. Several cellular kinase inhibitors including Raf-1 inhibitor GW5074 (Fig. 3D) [72] and the selective PI4KIIIβ inhibitor PIK93 (Fig. 3D) [71] also inhibit enterovirus replication. Despite the fact that these compounds are structurally unrelated, they all share a common resistance mutation profile that is comparable to that of enviroxime, suggesting a related mechanism of action [67]. Despite this common resistance profile, only some of the so-called enviroxime-like compounds target PI4KIIIβ; others on the contrary may have targets different from PI kinases that still need to be identified [71]. In summary, the indispensable function of 3A in several virus and/or host cell interactions (which will be discussed below in more detail) makes this protein a very attractive (indirect) target in the search for a chemotherapeutic agent.

*2.3.3. Targeting 3D protein*

Enteroviruses encode an RNA-dependent RNA polymerase (RdRp), also referred to as $3D^{pol}$, that functions in concert with several cellular and viral proteins as the catalytic unit in the synthesis of both plus- and minus strand viral RNA. As viral polymerases are the key component of genome replication and human host cells are devoid of such RdRps, they are considered one of the most interesting targets for drug development. The identification of numerous inhibitors targeting the HIV reverse transcriptase and HBV and herpes virus DNA polymerase has already clinically validated viral polymerases as excellent therapeutic targets. Inhibitors targeting the RdRp of HCV are currently in clinical development [73]. Antiviral drugs targeting $3D^{pol}$ may either target the enzymatic function of the protein itself, or inhibit interactions with viral and/or cellular proteins or with the RNA template. Polymerase-targeting compounds can be classified into nucleoside and non-nucleoside inhibitors. One such nucleoside inhibitor is the synthetic purine analogue 1-β-D-ribofuranosyl-1-H-1,2,4-triazole-3-carboxamide (ribavirin) (Fig. 3C) which has been used over several decades to treat viral infections, mostly HCV, in combination with pegylated IFN-α [74]. Despite the fact that the compound has been a topic of intensive research since its discovery in 1972, the precise molecular mechanism by which ribavirin exerts its antiviral activity still remains controversial. Several different mechanisms of action have been proposed depending on the virus being studied, including (i) depletion of guanosine triphosphate (GTP) pools via inhibition of inosine 5′-monophosphate dehydrogenase (IMPDH), (ii) maintaining an immunomodulatory response, (iii) inhibition of viral capping, (iv) direct inhibition of viral polymerases and finally (v) by inducing lethal mutagenesis in which ribavirin will be incorporated in the viral genome. This latter will increase the viral mutation rate and eventually force the viral population beyond the error threshold and, potentially, extinction [75,76]. Recent studies with poliovirus demonstrate that lethal mutagenic treatment does not only effectively reduce viral fitness, but results also in ribavirin-resistant virus populations with increased polymerase fidelity. This lowered mutation rate impaired viral spread, pathogenesis and capacity of evolving resistance against antiviral drugs targeting structural or nonstructural proteins [77]. Combination therapy, consisting of lethal mutagenesis-inducing agents and the classic antiviral approach could thus create an indirect synergism [78]. Only a few non-nucleoside anti-enterovirus agents have been identified so far including gliotoxin, amiloride and DTriP-22. Gliotoxin, a fungal metabolite, was shown to inhibit poliovirus replication by targeting $3D^{pol}$, thereby hampering the synthesis of both plus- and minus-strand RNA synthesis. Amiloride (Fig. 3D)

and its derivatives are known inhibitors of epithelial $Na^+$ channels and are used to treat hypertension. They also have been reported to inhibit rhinovirus 2 and CVB3 replication by targeting $3D^{pol}$ [79]. A recent study of Levi et al. suggested that amiloride acts as a non-nucleoside mutagenic agent by mediating alterations in intracellular ion levels such as $Mg^{2+}$ and $Mn^{2+}$. As these ions are essential cofactors for polymerase activity and nucleotide insertion, they affect RdRp fidelity in an indirect way and prove how lethal mutagenesis can be induced by targeting the cellular environment [80]. Finally, another non-nucleoside inhibitor, DTriP-22, was identified as an inhibitor of several enteroviruses. Generation of drug-resistant variants revealed that $3D^{pol}$ is the antiviral target. It was suggested that DTriP-22 impairs $3D^{pol}$ activity by hindering entry of nucleoside triphosphates into the cavity, but not by incorporation into newly formed RNA [81]. In summary, $3D^{pol}$ has a very specific and indispensible role in the viral life cycle, and is therefore considered as a prime target for antiviral drug development. Identifying inhibitors of $3D^{pol}$ is not only useful in a classical therapeutic approach but also provides very valuable tools to shed a light on viral evolution, genetic diversity and adaptation of virus populations in vivo.

### 2.4. Inhibitors that target morphogenesis

Despite the fact that morphogenesis of enteroviruses is rather poorly understood, it is known to be a multi-tiered process before infectious particles are formed. First, the capsid precursor P1, including the four structural components VP1 to VP4, is released from the polyprotein [82]. Upon release, the enterovirus capsid precursor interacts with chaperone heat shock protein 90 (Hsp90), probably in cooperation with Hsp70 and several cofactors, maintaining the nascent P1 in a processing-competent conformation and shielding it from proteasome degradation [83]. Subsequently, P1 is processed by $3C^{pro}$ or $3CD^{pro}$ yielding an immature structural unit, the protomer particle, containing one copy of VP0 (precursor of VP4 and VP2), VP3 and VP1 [84]. Five of these protomers will self-assemble into a pentamer; twelve of these pentamers form a next-higher-order empty capsid or procapsid. Recent studies on enterovirus morphology suggest a role of $2C^{ATPase}$ in the morphogenesis of virus particles by direct interactions with pentameric units via capsid protein VP3. In this way, pentamer particles will only condense around actively replicating genomic RNA forming a provirion [53,85]. Despite the fact that morphogenesis is a stepwise event with many potential targets, so far only few inhibitors were found to inhibit one of these steps in the final stage of virus replication. The replication of poliovirus and coxsackievirus A21 was shown to be inhibited by 5-(3,4-dichlorophenyl)methylhydantoin by interference with the correct encapsidation of viral RNA and with post-synthetic cleavages of poliovirus proteins [86,87]. Geldanamycin (Fig. 3D), a specific inhibitor of the cellular protein chaperone Hsp90 was shown to impair the correct folding and maturation of enterovirus capsid proteins [83]. In summary, morphogenesis is a stepwise process involving both cellular and viral factors and is a critical step in the release of infectious virus particles. Future research should allow further differentiation of this multi-tiered process through the identification of critical cellular and viral factors that can be targeted by a specific inhibitor.

### 2.5. Inhibitors that target host cell factors

Because of the quasispecies nature of enteroviruses, the rapid emergence of drug resistance remains a major concern with the current antiviral approach based on small molecular inhibitors [88]. This calls for new generations of inhibitors. Although targeting virus-encoded proteins will likely remain the major therapeutic approach, virus replication can also be inhibited by targeting host cell factors that are essential for viral replication. Viral proteins require and interact with the cellular machinery to facilitate genome replication, translation and even morphogenesis. A potential advantage of such approach is the fact that cellular factors may be less prone to drug resistance due to their genetic stability. Combination therapies based on targeting both viral and cellular factors may prevent or delay the emergence of drug-resistant variants [71,83,88]. Although several studies demonstrated the interplay between virus and host factors, so far only few cellular proteins have been identified as a possible antiviral target. Geldanamycin (Fig. 3D), and its pharmacologically more active analogue 17AAG, acts as a potent inhibitor of enterovirus replication. Geldanamycin, a known inhibitor of chaperone Hsp90, impaired the correct folding and maturation of enterovirus capsid proteins, without any apparent toxicity in vivo. This low toxicity could be explained by the fact that partial inhibition of a single chaperone system does not impair cellular protein folding which is distributed among many chaperones. In addition, despite the high mutation rate, poliovirus was not capable of developing resistance against geldanamycin, revealing a potential target for the development of antiviral therapies that may be refractory to drug resistance [83]. As described above, PI4KIIIβ has recently been identified as an essential component of enterovirus replication [69]. Several molecules have been described that target this kinase including GW5074 (Fig. 3D), PIK93 (Fig. 3D) and T-00127-HEV1 [71]. Despite the fact that also in this case the virus was still able to develop resistance to the inhibitor, the drug-resistant variants were shown to have a reduced viral fitness, suggesting that ablation of virus replication could render the virus more responsive to the host's immune response or therapy with another agent that targets another component in the replication cycle [71]. In summary, targeting host factors that are critical to viral replication (but whose action can be bypassed via other factors in the host cell biology) may form attractive targets for inhibition of viral replication. Understanding the complex virus–host interactions holds great promise for developing new approaches to combat enterovirus infections and will also provide better insights into the replication of these viruses.

## 3. Conclusion

Many molecules that block in vitro enterovirus replication have been reported in the preceding decades. Although these compounds were designed with the aim to eventually treat infected patients, none of them got final approval for marketing. In many cases, toxicity or poor pharmacokinetics posed major issues. On the other hand, the clinical applications for such drugs called for very few if not any adverse effects. Indeed, a treatment for the common cold should not go along with adverse effects that are similar than, or worse than the symptoms of the common cold itself. Similarly, treatment of children and neonates (e.g. for enterovirus sepsis) requires a drug that is extremely safe and well-tolerated. Nowadays, the pressure to develop anti-enterovirus drugs has augmented and hence, the constraints for such drugs in term of tolerability might be reconsidered. Epidemics among children are reported increasingly and often lead to fatal outcomes. Especially in Asia, enteroviruses have become a major threat in the young population and clinicians urgently call for an efficient and selective treatment. Likewise, the need for a treatment to block rhinovirus infections has become more urgent since it is now widely recognized that these viruses are implicated in exacerbations of chronic lung diseases. As the clinical relevance of enteroviruses has become more serious, the demands as well as the efforts in the field are accelerating. We therefore expect that the question is no longer if but when an anti-enterovirus drug will

appear on the market. Although antivirals and vaccines are the two principle weapons to combat viral infections, we believe that vaccines have an incomplete role in the fight against enteroviruses. Considering the rhinoviruses, the number of serotypes reported so far exceeds 100. For polioviruses, the use of OPV will be discontinued in the post-eradication era, whereas the IPV alone is insufficient to provide complete protection in possible future outbreaks. It is unmistakable that the search for novel, potent and selective therapies to tackle enterovirus infections is one of the major challenges for the years to come in the field of antivirals.

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