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Review Article

Valinomycin as a potential antiviral agent against coronaviruses: A review

Dong Zhang, Zhi Ma, Hanchi Chen, Yuele Lu, Xiaolong Chen**

Institute of Fermentation Engineering, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou, PR China


Dr. Xiaolong Chen


Dr. Yuele Lu

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ABSTRACT

Human coronaviruses (HCoVs), including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have been resulting in global epidemics with heavy morbidity and mortality. Unfortunately, there are currently no specific medicines that can better treat these coronaviruses. Drug repurposing is an effective and economical strategy for drug discovery from existing drugs, natural products, and synthetic compounds. In this review, the broad-spectrum antiviral activity of valinomycin (VAL), especially its activity against coronaviruses such as SARS-CoV, MERS-CoV, human coronavirus OC43 (HCoV-OC43), were summarized, it highlights that VAL has tremendous potential for use as a novel antiviral agent against SARS-CoV-2.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), provisionally named 2019 novel coronavirus (2019-nCoV), is the causal agent of the outbreak of coronavirus disease 2019 (COVID-19) [1,2]. Coronaviruses (CoVs) are a group of enveloped viruses with a single-strand, positive-sense RNA genome ranging from approximately 26 to 32 kilobase [3]. Its genome organization for CoVs is similar: about two-thirds of the 5′-proximal genome contains the ORF1a/b replica gene, and the remainder encodes the spike, envelope, membrane, nucleocapsid structural proteins, and several accessory proteins [4]. Coronaviruses are divided into four genera: alphacoronaviruses (α-CoVs), betacoronaviruses (β-CoV),

* *Corresponding author*. Institute of Fermentation Engineering, College of Biotechnology and Bioengineering, Zhejiang University of Technology, 18, Chaowang Rd., Hangzhou 310014, China.
E-mail addresses: luyuele@zjut.edu.cn (Y. Lu), richard_chen@zjut.edu.cn (X. Chen).
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gammacoronaviruses (γ-CoVs), and deltacoronaviruses (δ-CoVs) [5]. They have the potential to cause respiratory, enteric, hepatic, and neurologic diseases among humans, other mammals, and birds [6,7]. In detail, only α-CoVs and β-CoVs can infect humans while most γ-CoVs and δ-CoVs can infect avian species. Since the first isolation of the coronavirus in 1937, seven coronaviruses were currently known to cause human disease, as shown in Table 1. In the mid-1960, two coronaviruses human coronavirus 229E (HCoV-229E) and human coronavirus OC43 (HCoV-OC43) were isolated from human [8–13]. Subsequently, the other five human coronaviruses (HCoVs) were identified: severe acute respiratory syndrome coronavirus (SARS-CoV) [14–16], human coronavirus NL63 (HCoV-NL63) [17–19], human coronavirus HKU1 (HCoV-HKU1) [20–22], Middle East respiratory syndrome coronavirus (MERS-CoV) [23,24], and SARS-CoV-2 [1,2]. HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 are prevalent and typically cause common cold symptoms in immunocompetent persons. However, SARS-CoV, MERS-CoV, and SARS-CoV-2 can cause severe acute respiratory syndrome and have subsequent person-to-person transmission [25–27]. SARS-CoV was the first coronavirus that caused an epidemic of severe acute respiratory syndrome (SARS), which was associated with an outbreak of atypical pneumonia [28]. The major clinical features include persistent high fever and mild respiratory symptoms but rapidly progressed to pneumonia [29–32]. According to vital statistics data from 2002 to 2003, SARS-CoV resulted in 8437 cases and 813 death in 29 countries [33]. Then, MERS-CoV was the causal agent of Middle East severe respiratory disease outbreaks, which plagued 2494 people and caused 858 death between 2012 and 2019 in 27 countries worldwide [34]. Data as of 18 May 2020, SARS-CoV-2 resulted in 4,534,731confirmed cases and 307,537 confirmed deaths in 216 countries, areas, or territories. The news about the new coronavirus disease (COVID-19) outbreak is ongoing updated by the World Health Organization (WHO) (www.who.int/emergencies/diseases/novel-coronavirus-2019). Though SARS-CoV-2 was a new human pathogen that causes severe respiratory illness, it's similar to MERS-CoV and SARS-CoV [1,35]. Currently, no specific drugs are available to target SARS-CoV-2. Fortunately, drug repurposing has been proved to be an effective and economical drug discovery strategy from existing anti-CoVs drugs [36,37]. Therefore, the inhibitors with broad-spectrum antiviral activity against HCoVs, especially MERS-CoV and SARS-CoV, may be potent drug candidates against SARS-CoV-2 [38,39].

Valinomycin (VAL) has broad-spectrum biological activities such as antitumor [40], antibacterial [41], antifungal [42], insecticidal [43], and antiviral activities [44,45]. It was first isolated from *Streptomyces fulvissimus* by Brockmann et al., in 1955 [46]. The structure of VAL was determined as 12 stereogenic centers consisting of a three repeating sequence of the tetramer $_{D}$-α-hydroxyisovaleric acid-$_{D}$-valine-$_{L}$-lactate-$_{L}$-valine ($_{D}$-Hiv-$_{D}$-Val-$_{L}$-Lac-$_{L}$-Val). VAL can selectively transport K^+ across model lipid bilayer membranes [47]. Furthermore, VAL is also a respiratory chain ionophore inhibitor that inhibits oxidative phosphorylation by increasing the permeability of the mitochondrial inner membrane to K^+ [48]. Total synthesis of valinomycin has been mentioned in the previous article [42,49–51]. On the other hand, the gene cluster of VAL has been characterized [44,52]. The biosynthesis pathway for VAL has been proposed, and as shown in Fig. 1 [52,53]. VAL is biosynthesized by a nonribosomal peptide synthetase (NRPS) called tetramodular valinomycin synthetase (VlmSyn), which is coded by two large NRPS genes with distinctive domain organization A-KR-T-C-A-T-E-C for *vlm1* (10,287 bp) and A-KR-T-C-A-T-TE for *vlm2* (7968 bp), and functionally defined small ORFs [44,53,54]. Moreover, VlmSyn is divided into four modules, which consist of domains with adenylation (A), ketoreductase (KR), thiolation (T), condensation (C), epimerase (E), and thioesterase (TE) functions. Each module assembles $_{D}$-Hiv, $_{D}$-Val, $_{L}$-Lac, or $_{L}$-Val to form a tetradepsipeptide basic unit. Besides, α-ketoisovalerate (α-Kiv), pyruvate, and $_{L}$-Val as basic precursors are needed. KR domain reduces α-Kiv to $_{D}$-Hiv in Module 1. E domain transfers $_{L}$-Val to $_{D}$-Val in Module 2. Pyruvate is reduced to $_{L}$-Lac *via* KR domain in Module 3. Besides, production levels of valinomycin in *Streptomyces* and *Escherichia coli* were summarized, as shown in Table 2 [41,53–67].

VAL, a cyclodepsipeptide antibiotic acting as a potassium ion transporter, was first considered as the most potent inhibitor of SARS-CoV among more than 10,000 drug candidates by Wu, Jan et al., in 2004 [45]. Then, researchers reported potential broad-spectrum activity of VAL against the other four human coronaviruses such as MERS-CoV, HCoV-OC43, HCoV-NL63. Furthermore, VAL showed broadly antiviral activities

Table 1 Seven human coronaviruses.

Human coronavirus	Year	Type	Host	Cellular Receptor	References
HCoV-229E	1965	α-CoV	Bats	HAPN	[8,11,12]
HCoV-OC43	1967	β-CoV	Cattle	9-O-Acetylated sialic acid	[9,10,13]
SARS-CoV	2003	β-CoV	Palm Civets, Bats	ACE2	[14–16]
HCoV-NL63	2004	α-CoV	Palm Civets, Bats	ACE2	[17–19]
HCoV-HKU1	2005	β-CoV	Mice	9-O-Acetylated sialic acid	[20–22]
MERS-CoV	2012	β-CoV	Camels, Bats	DPP4	[23,24]
SARS-CoV-2	2019	β-CoV	Pangolin, Bats	ACE2	[1,2]

Abbreviations HCoV: Human coronavirus; SARS-CoV: Severe acute respiratory syndrome coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; α-CoV: alphacoronaviruses; β-CoV: betacoronaviruses; HAPN: Human Aminopeptidase N; ACE2: Angiotensin-converting enzyme 2; DPP4: Dipeptidyl peptidase 4.

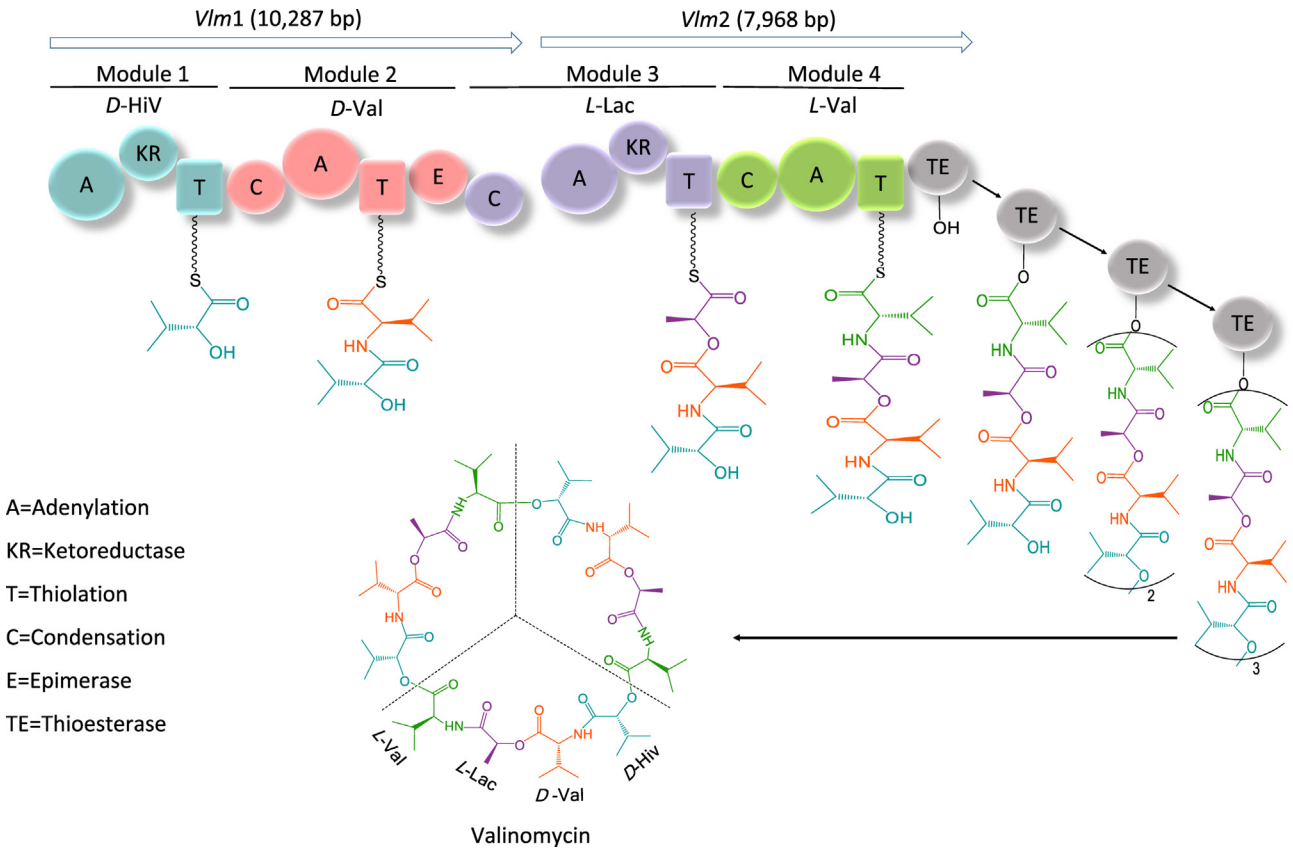

Fig. 1 Proposed pathway for the biosynthesis of valinomycin.

against other viruses, including one subfamily *Coronavirus* mouse hepatitis virus A59 (MHV-A59), vesicular stomatitis virus (VSV), poliovirus (PV), hepatitis B virus (HBV), enteroviruses (coxsackievirus B3 and human rhinovirus 2), porcine reproductive and respiratory syndrome virus (PRRSV), respiratory syncytial virus (RSV), Lassa virus (LASV), lymphocytic choriomeningitis mammarenavirus (LCMV), La Crosse virus (LACV), Rift Valley fever virus (RVFV), Keystone virus (KEYV), and Zika virus.

This review summarizes the broad-spectrum antiviral activities of VAL against nineteen viruses, including HCoV-229E, HCoV-OC43, HCoVNL63, SARS-CoV and MERS-CoV. And the mechanism of VAL on viruses was discussed. Furthermore, it demonstrated that VAL may be developed as an antiviral therapeutic agent with the potential to combat fast-spreading coronavirus disease 2019 (COVID-19).

Antiviral activity of valinomycin against human coronaviruses

In this section, we describe the antiviral activity of VAL against five HCoVs, including SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-NL63, HCoV-229E. The antiviral activities of VAL against coronaviruses are shown in Table 3.

SARS-CoV

SARS-CoV was the causal agent of the severe acute respiratory syndrome (SARS) epidemic, which was caused by a laboratory accident or probably transmitted to humans by animals [15,16]. SARS-CoV enters its host cell by the Angiotensin-

Table 2 Production levels of valinomycin.

Strains	Yield ($mg \cdot L^{-1}$)	References
Streptomyces tsusimaensis (ATCC 15141)	8.45	[54–56]
Streptomyces sp. PRL 1642 (ATCC 23836)	23.19	[54,55,57,58]
Streptomyces anulatus (Montana)	24.68	[41,54,55]
Streptomyces anulatus (Malaysia)	25.22	[41,54,55]
Streptomyces exfoliatus (Malaysia)	32.78	[41,54,55]
Streptomyces fulvissimus (DSM 40767)	4.25	[54,55,59]
Streptomyces griseus 1/k (DSM 41748)	10.19	[54,55,60]
Streptomyces griseus 10/ppi (DSM 41751)	22.08	[54,55,60]
Streptomyces sp. M10	3.83	[61]
Streptomyces lavendulae (ACR-DA1)	19.4	[62]
Streptomyces lavendulae (ACR-DA1)	84	[63]
Streptomyces sp. P11–23B	0.736	[64]
Escherichia coli BJJ01	6.4	[65]
	2	[66]
	3.3	[67]
	13	[53,55]

Table 3 Antiviral activity of valinomycin.

Coronaviruses		Activities (μM)	References
Human coronaviruses	SARS-CoV	$EC_{50} = 0.85$ $CC_{50} = 68$	[45]
	MERS-CoV	$EC_{50} = 6.07$ $CC_{50} = 5.88$	[68,70]
	HCoV-OC43	$EC_{50} = 4.43$ $CC_{50} = 6.15$	[70]
	HCoV-NL63	$EC_{50} = 1.89$ $CC_{50} = 4.12$	[70]
	HCoV-229E	$IC_{50} = 0.067$	[71]
Other viruses	VSV	$EC_{90} = 10$	[41]
	CVB3	$IC_{50} = 0.971$	[71]
	HRV2	$IC_{50} = 0.61$	[71]
	PRRSV	$IC_{50} = 0.024$	[86]
	RSV	$IC_{50} = 0.0015$ $CC_{50} = 2.705$	[82]
	MHV-A59	$EC_{50} = 6.78$ $CC_{50} = 5.11$	[70]
	LASV/vRNP	$EC_{50} = 0.61$ $CC_{50} = 1.22$	[90]
	LCMV/vRNP	$EC_{50} = 0.15$ $CC_{50} = 1.93$	[90]
	LACV	$IC_{50} = 0.588/0.898$ $CC_{50} = 14.7$	[71]
	RVFV	$IC_{50} = 0.041$	[71]
	KEYV	$IC_{50} = 0.156$	[71]
	ZIKV	$IC_{50} = 0.078$	[71]

Abbreviations EC_{50}/EC_{90}: 50%/90% effective concentration; IC50: 50% inhibitory concentration; VSV: Vesicular stomatitis virus; PRRSV: Porcine reproductive and respiratory syndrome virus; CVB3: Coxsackievirus B3; HRV2: Human rhinovirus 2; RSV: Respiratory syncytial virus; MHV-A59: Mouse hepatitis virus A59; LACV: La Crosse virus; LASV: Lassa virus; LCMV: Lymphocytic choriomeningitis mammarenavirus; vRNP: Virus ribonucleoprotein. LACV: La Crosse virus; RVFV: Rift Valley fever virus; KEYV: Keystone virus; ZIKV: Zika virus.

converting enzyme 2 (ACE2) receptor, which is a member of the renin-angiotensin system. Besides, its genome organization is similar to that of previously known coronaviruses, for example, HCoV–229E and HCoV–OC43 [14,16]. The development of drugs against SARS-CoV was significant and necessary. The activity of the valinomycin against SARS-CoV was reported by Wong's group at Academia Sinica, Taiwan [45]. During multiple concentrations, the 50% effective concentrations for the inhibition of viral replication (EC_{50}, 0.85 μM) and host growth (CC_{50}, 68 μM) were determined by a cell-based assay using SARS virus and Vero E6, and used to evaluate the activity of VAL. The results indicated that VAL exhibited the most potent antiviral activity against SARS-CoV among nearly 10,000 antiviral agents, including existing drugs, natural products, and synthetic compounds. However, the mode of action of VAL against SARS-CoV is not yet known [44].

MERS-CoV

MERS-CoV, formerly known as a novel coronavirus (NCoV), was identified as the etiological agent of the Middle East respiratory syndrome (MERS), which causes a severe respiratory illness with symptoms of fever, cough, and shortness of breath [23]. Since 2012, the WHO has reported 2,494 laboratory-confirmed cases of infection with MERS-CoV, of which 858 MERS-CoV associated deaths have occurred in 27 countries (https://www.who.int/emergencies/mers-cov/en/). In 2019, German scientists found that E3 ligase S-phase kinase-associated protein 2 (SKP2) executes lysine-48-linked polyubiquitination of Benclin 1 (BECN1), leading to its proteasomal degradation. SKP2-BECN1 Link is a potent target for host-directed antiviral drugs and other autophagy-sensitive diseases. Notably, VAL is considered to act as an SKP2 inhibitor, which enhances BECN1 protein stability and autophagy and efficiently reduces the replication of MERS-CoV [68,69]. VAL inhibited MERS-CoV replication by up to 1,000-fold at 48 h post-infection at a concentration of 10 μM, while it enhanced ATG14 oligomerization about 2-fold and increased the number of autolysosomes by > 2-fold [68]. At the same time, National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China, also revealed that VAL inhibited the replication of MERS-CoV with an EC_{50} value of 6.07 μM and CC_{50} value of 5.88 μM using an experimental design of dose–response studies *in vitro* [70]. Recently, it demonstrated that VAL exhibited inhibitory activity with an IC_{50} value of 0.005 μM [71].

HCoV-OC43

HCoV-OC43 is a respiratory epithelial virus, belonging to genus Betacoronavirus, subgenus *Embecovirus* of the family *Coronaviridae*, which widely spread in the population and is related to the common cold [72]. Compared with spike (S) gene sequences of HCoV-OC43 and bovine coronavirus (BCoV), which is another host-type of betacoronavirus that causes respiratory disease, calf diarrhea, and winter dysentery in cattle, it was proposed that bovine-to-human spillover of BCoV leaded to HCoV-OC43 infection [73]. 9-O-acetylated sialic acids are the cellular receptor of HCoV-OC43, as well as HCoV-HKU1 [13]. In 2019, the effect of VAL on HCoV-OC43 was reported by China CDC, Beijing, in 2019 [70]. VAL inhibited the replication of HCoV-OC43 with an EC_{50} value of 4.43 μM and a CC_{50} value of 6.15 μM. The mechanism of action VAL against HCoV-OC43 is not yet reported.

HCoV-NL63

HCoV-NL63 is an enveloped positive-sense single-stranded RNA virus that was first isolated from a seven-month-old child with severe lower respiratory tract in Amsterdam in 2004 [17,18]. Same as SARS-CoV and SARS-CoV-2, HCoV-NL63 enters its host cell by binding to the ACE2 receptor [19].

HCoV-NL63 can cause mild upper respiratory illness with symptoms of cough, fever, or rhinorrhea, or result in more serious lower respiratory tract infections with symptoms of bronchiolitis and croup [74,75]. Along with HCoV-OC43, HCoV-NL63 are common causes of upper respiratory tract infections, which occur more frequently than HCoV-HKU1 and HCoV-229E infections in early childhood [76–78]. VAL showed inhibitory activity against HCoV-NL63 with an EC_{50} value of 1.68 μM and a CC_{50} value of 4.12 μM [70]. The mechanism of action of VAL against HCoV-NL63 has not been investigated.

HCoV-229E

HCoV-229E is the first identified human coronavirus that belongs to a member of the genus *Alphacoronavirus* and subgenus *Duvinacovirus* [12]. HCoV-229E and HCoV-OC43 are alphacoronaviruses among the seven known human coronaviruses, while the other five human coronaviruses are betacoronavirus. HCoV-229E, along with HCoV-OC43, were considered the cause of the virus that causes the common cold with the symptoms of lower respiratory tract infections and otitis media [76]. The cellular receptor of HCoV-229E is Human Aminopeptidase N (HAPN) [11]. During the rapid antiviral screening to identify potential antivirals against the La Crosse virus, the VAL active against HCoV-229E infection was reported by Sandler et al. [71]. VAL showed inhibitory activity with an IC_{50} value of 0.067 μM and exhibited a potent inhibitory against the replication of HCoV-229E at the concentration of 10 μM. The mechanism of VAL against HCoV-NL63 was unclear.

Antiviral activity of valinomycin against other viruses

Vesicular stomatitis virus

Vesicular stomatitis virus (VSV) is an enveloped negative-sense single-stranded RNA virus that belongs to the genus *Vesiculovirus* of the family Rhabdoviridae [79,80]. VSV is the primary cause of vesicular disease outbreaks in livestock. In 1999, the antiviral activity of VAL against VSV was determined [41]. Specific infectivities of partially purified ^{35}S-methionine-labeled VSV had no apparent changes in the presence and absence of VAL. The reduction in VSV titer in the presence of VAL may be due to a reduction in the production of virus particles, in comparison with the releasing of noninfectious particles. VAL affected the processing of glycoprotein (G protein). In the presence of VAL, G protein oligosaccharides were sensitive to endo-b-N-acetylglucosaminidase H, which can cleave high-mannose oligosaccharides but not complete processing of complex oligosaccharides. As a result, most of the oligosaccharides in G protein were not converted into VSV G protein with mature structure and function required for transport of G protein to the cell surface and its further incorporation into budding particles. The addition of 10 μM VAL to the infected Vero cells within the first 3 h, resulted in a 90% reduction in viral titer 12 h after infection. Of course, higher concentrations of valinomycin resulted in an even greater reduction in viral titer.

Poliovirus

Poliovirus (PV) is a non-enveloped single-stranded positive-sense RNA virus that belongs to the species *Enterovirus C* of family *Picornaviridae*. There are three wild types of poliovirus (PV)—PV-1, PV-2, and PV-3. PV spreads from person to person and can cause devastating epidemics of poliomyelitis. Though two vaccines were used to protect against poliovirus infection, the infectious entry route of PV is still unclear (https://www.cdc.gov/cpr/polioviruscontainment/diseaseandvirus.htm). According to Irurzun et al. [81], although poliovirus does not require an intact pH to enter into the susceptible cell, its RNA requires an intact concentration of K^+ inside the cells to be uncoated and to enter the cytoplasm. The productive poliovirus entry of poliovirus is blocked by and concanamycin A. In other words, VAL exerted inhibitory effect at the stage of poliovirus replication [82]. The replication of poliovirus was powerfully inhibited by the combination of a vacuolar proton-ATPase inhibitor concanamycin and ionophore antibiotics VAL at the concentration of 80 nM and 50 μM, respectively [81].

Hepatitis B virus

Hepatitis B virus (HBV) is an enveloped partially double-stranded DNA virus that belongs to the genus *Orthohepadnavirus* of the family *Hepadnaviridae*. HBV was identified as the causative agent of the disease hepatitis B, which is a major cause of global health problems. It is a viral infection that can cause both acute and chronic infection of the liver. Millions of people are infected with chronic hepatitis B annually and led to approximately 900,000 deaths in 2005 (https://www.who.int/news-room/fact-sheets/detail/hepatitis-b). The interaction of hepatitis B virus (HBV) polymerase (Pol) with Ca^{2+}-modulated protein S100A10 (p11) is important for performing multiple functions required for viral replication. The activity of VAL against HBV was evaluated through exploring effects of Ca^{2+} on the nuclear localization of HBV Pol and p11 in HepG2 cells, which were incubated within 30 μM VAL for 24 h. The results showed that VAL promoted Ca^{2+} influx, resulted in an inhibition of the association of HBV Pol—p11 with the promyelocytic leukemia protein PML. In other words, VAL showed anti-HBV activity in viral replication and transcription [83].

Enteroviruses

Enterovirus is a genus of a non-enveloped single-stranded and positive-sense RNA virus that belongs to the members of the *Picornavirus* family. More than 90 subtypes of enteroviruses have been identified, including rhinovirus and Coxsackievirus B3 (CVB3). CVB3 is one of the major pathogens that may cause hand, foot, and mouth disease (HFMD), as well as disease of muscles, lungs, and heart in infants and young children. Human rhinovirus (HRV) is the most common viral infectious agent in humans and is the major cause of the common cold, which causes billions of dollars losses annually in higher medical costs and lost productivity at work [84]. Berka et al.

reported that HRV2 type 2 (HRV2) uncoating and RNA translocation are not affected by a pH gradient, but are affected by membrane potential between the acidic endosome lumen and the neutral cytoplasm [85]. VAL depolarized the host membrane, thereby preventing the fusion of the HRV2 with the endosomal membranes. Besides, VAL had a slight inhibitory effect on the synthesis of cellular protein, and the results were only shown when compared with other drugs [82,85]. Sandler et al. demonstrated that VAL exhibited inhibitory activity against CVB3 and HRV2 during culture tests on Huh7 cells with IC_{50} values of 0.971 μM and 0.61 μM, respectively [71].

Porcine reproductive and respiratory syndrome virus

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped positive-sense single-strand RNA virus that causes porcine reproductive and respiratory syndrome (PRRSV). It belongs to the genus *Arterivirus* of the family *Arteriviridae*. Since its first emergence in the USA in 1987 and Europe in 1990, PRRSV causes late-term reproductive failure in breeding severe stock pneumonia in neonatal pigs in swine worldwide (https://www.prrs.com/en/prrs/). Karuppannan et al. established a high-throughput screening method to screen potent agents inhibiting the replication of PRRSV in a library of 502 purified natural compounds. VAL is known to act on ion channels of cells membrane. It did not block the binding and entry steps of the PRRSV but took action during the subsequent virus replication process [86]. VAL was described as one of the most potent specific inhibitors of the PRRSV replication with IC_{50} = 24 nM in infected MARC-145 cells.

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is an enveloped negative-sense single-stranded RNA virus that usually causes mild and cold-like symptoms (https://www.cdc.gov/rsv/index.html). RSV is a leading cause of lower respiratory tract infections in infancy and childhood worldwide. Though ribavirin is used for clinical therapy, there is no effective vaccine against RSV. According to Norris et al. [82], the cardiac glycosides were identified as inhibitors of the membrane-bound Na^+/K^+-ATPase against the replication of RSV. Notably, VAL has a high selectivity for intracellular K^+ relative to Na^+ [47]. This mechanism of action of VAL on RSV at this stage of the replication cycle is the same as that of poliovirus. Therefore, VAL exerted antiviral activity at a post-entry stage, mainly affecting the viral transcription and replication stages of the viral life cycle [81,82]. Therefore, the VAL was screened out as an anti-RSV inhibitor in human epithelial type 2 cells and primary nasal epithelial cells RSV with an IC_{50} value of 0.0015 μM and a CC_{50} value of 2.705 μM [82].

Mouse hepatitis virus A59

Mouse hepatitis virus A59 (MHV-A59) is an enveloped positive-sense single-stranded RNA virus that belongs to a member of the genus *Betacoronavirus* within the subfamily *Coronavirinae*. It caused a variety of syndromes in susceptible strains of mice, including hepatitis, thymus involution, and hypergammaglobulinaemia [87]. It was reported that the genomes of the MHV-A59 display 71% identity with two HCoV-OC43 variants, which were obtained from the American Type Culture Collection (ATCC) and a clinical isolate [88]. The cellular receptor of MHV-A59 is Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) (CEACAM1) [89]. Later, China CDC et al. reported that VAL inhibited the replication of MHV-A59 at the concentration of EC50 = 6.78 μM and CC_{50} = 5.11 μM [70]. However, the researchers have not further studied the mechanism of VAL against MHV-A59.

Lassa virus and lymphocytic choriomeningitis virus

Lassa virus (LASV) is an enveloped single-stranded segmented ambisense RNA virus that causes Lassa hemorrhagic fever, which is a highly prevalent febrile disease associated with high morbidity and significant mortality in West Africa (https://www.cdc.gov/vhf/lassa/). Lymphocytic choriomeningitis mammarenavirus (LCMV) was the initially isolated arenavirus that belongs to a member of the family *Arenaviridae* in 1933. LCMV was identified as the causative agent of lymphocytic choriomeningitis (LCM), which is associated with aseptic meningitis, encephalitis, or meningoencephalitis (https://www.cdc.gov/vhf/lcm/index.html). Virus ribonucleoprotein (vRNP) is responsible for directing viral RNA genome replication and gene transcription of LASV and LCMV. Cubitt et al. established a high throughput screen to identify VAL, which was an effective inhibitor of the activity of LASV/vRNP and LCMV/vRNP in the cell-based, infectious-free, platform. VAL exhibited strong inhibitory effect on LCMV and LASV vRNP activity with EC_{50} = 0.61 μM (CC_{50} = 1.22 μM), and EC_{50} = 0.15 μM (CC_{50} = 1.93 μM), respectively [90].

Bunyaviruses

Bunyaviruses are spherical or pleomorphic, enveloped viruses that infect humans and cause rashes and fever and even encephalitis. Bunyaviruses belong to the family *Bunyaviridae*, including La Crosse virus (LACV), Rift Valley fever virus (RVFV), Keystone virus (KEYV), and so on. These viruses are transmitted to humans through bites or contact with the blood or tissues of infected animals. LACV is a California serogroup bunyavirus that can cause encephalitis or inflammation of the brain, but no specific drugs target the LACV. RVFV is an enveloped negative single-stranded RNA virus that causes Rift Valley fever (RVF), which is a viral zoonosis that affects animals and can infect humans. KEYV is a mosquito-borne virus that can cause humans with minor symptoms of a rash and fever. In other words, Bunyaviruses can infect humans and cause rashes and fever and even encephalitis. Sandler et al. established a rapid antiviral screening method to screen several potential antiviral molecules, including known and novel antivirals, which exhibited antiviral activity against LACV [71]. According to Mankouri et al. [91], it demonstrated that the activity of cellular K^+ channels is necessary to cause productive infection of several bunyaviruses, and K^+ channels as targets to impede virus entry, infection, and disease. Besides, Sandler et al. reported that VAL does not reduce viral particle infectivity and the ability of virus binding to host cells,

but may preclude virus replication by altering cellular K^+ gradient. In other words, VAL exerted antiviral activity at the entry stages of bunyaviruses infection within host cells [71]. VAL potently inhibited LACV replication with an IC_{50} value of 0.588 μM and 0.898 μM during culture tests on Huh7 cells and Vero-E6 cells, respectively. Moreover, Treatment with the VAL resulted in a viral titer reduction in the replication of RVFV and KEYV with IC_{50} values of 0.041 μM and 0.156 μM, respectively [71].

Zika virus

Zika virus (ZIKV) is an enveloped positive-sense single-stranded RNA virus that belongs to the genus *Flavivirus of* the family *Flaviviridae*. ZIKV was first identified from monkeys in 1947 and from humans in 1952. ZIKV is mainly transmitted by the bite of mosquitoes and causes an outbreak of mild symptoms, which is similar to a very mild form of dengue fever (https://www.who.int/news-room/fact-sheets/detail/zika-virus). Unfortunately, there is no specific medicine and vaccines for ZIKV infection. Recently, the outbreak of ZIKV disease has been reported evidence of mosquito-transmitted Zika infection in a total of 86 countries and territories. Although there is no specific report on the mechanism of VAL on ZIKV, Sandler et al. also mentioned that potassium ionophore VAL precluded the ZIKV infection by altering cellular K^+ gradient, which is a conserved and vital host factor in virus replication. ZIKV was sensitive to VAL, and ZIKV infection was restricted completely with above 0.5 μM VAL treatment. Besides, VAL had activity against ZIKV with an IC_{50} value of 0.078 μM [71].

Conclusion and prospect

The studies have shown that LD50 of VAL given i.p. in mice was 1.7 mg/kg, LD50 for liposome incorporated valinomycin (MVL-VM) was more than 50 mg/kg. LD50 of VAL form given i.v. is shown to be 0.18 mg/kg, where the LD50 for MLV-VM passed through a 0.6-μm filter was greater than 10 mg/kg [92]. Likewise, the LD50 of VAL is shown to be 0.98 and 4.14 mg/kg, when given to mice by intraperitoneal and subcutaneous injection, respectively [93]. However, the cytotoxicity test using Vero E6 cells indicated that VAL is non-inhibitory to Vero E6 at concentrations higher than four times the anti-SARS concentrations [45]. Furthermore, Sandler et al. discovered that no significant cellular toxicity was observed either by measuring gross cellular morphology or cellular ATP levels when the concentration of VAL was below 10 μM. The effective doses of VAL against the replication of viruses are often less than 10 μM (Table 3). Namely, VAL has antiviral effects in a variety of cell types at non-cytotoxic doses and reduces virus titers and cell-associated virus genomes [71]. Despite VAL is not an FDA-approved antiviral drug for human, modification of the structure of VAL may reduce drug's toxicity while maintaining antiviral activity *in vivo*. In other words, it is necessary to further study the antiviral activity and drug's safety of synthesized derivatives and analogs of VAL in animal models or human clinical trials [94,95].

On January 30, 2020, WHO declared the outbreak of COVID-19 a Public Health Emergency of International Concern (PHEIC) and made a series of temporary recommendations. Due to the lack of antiviral therapies and vaccines, the main treatment strategy for COVID-19 is supportive care, supplemented by broad-spectrum antibiotics, antivirals, corticosteroids, nucleoside analogues, protease inhibitors, and recovery plasma, and so on [96]. Recently, it demonstrated that remdesivir and chloroquine exhibited potential antiviral activity against SARS-CoV-2 during culture tests on Vero E6 cells with 50% effective concentrations of 0.77 μM and 1.13 μM, respectively [97]. Of note, the genome of SARS-CoV-2 shares 79.6% and 50% sequence identity to SARS-CoV and MERS-CoV, respectively, and is 96% identical at the whole-genome level to a bat coronavirus [2,35]. VAL was remained as the most potent inhibitor for the replication of SARS-CoV with a value of $EC_{50} = 0.85$ μM and $CC_{50} = 68$ μM using a cell-based assay.

Furthermore, this review summaries a list of nineteen viruses with corresponding EC50 and IC50 to VAL, and five of these viruses were HCoVs, including HCoV-229E, HCoV-OC43, HCoVNL63, SARS-CoV, and MERS-CoV. Although the mechanism of VAL against certain viruses has not been reported, such as SARS-CoV, HCoV-OC43, and HCoV-NL63, its mechanism on some specific viruses has been described in the relevant summary. VAL exhibited antiviral activity against non-enveloped viruses such as poliovirus and HRV2. Studies on the HRV2 have shown that VAL depolarized the host membrane to block viral fusion with the endosomal membranes and had a slight inhibitory effect on the synthesis of cellular protein. VAL showed an inhibitory effect at the replication phase of the poliovirus life cycle. It is worth noting that this mechanism of VAL on the non-enveloped virus poliovirus is the same as that against enveloped virus RSV. Also, VAL has shown other mechanisms against the replication of enveloped viruses. For instance, VAL act as an inhibitor SKP2 that enhances autophagy effectively and reduces the replication of MERS-CoV. VAL inhibited the replication of VSV by affected the processing of G protein. Specifically, in the presence of VAL, most of the oligosaccharides in VSV G proteins were not converted into structurally and functionally mature form, which is required for transport of G protein to the cell surface and its further incorporation into budding particles. VAL also inhibited the activity of LCMV and LASV VRNP, which are responsible for directing viral RNA genome replication and gene transcription. In addition, the ionophore antibiotic VAL disrupted the K^+ gradient that is a conserved and vital host factor in virus replication, leading to abnormal cellular events, including endocytosis required for efficient virus entry. These findings indicate that VAL may be repurposed as an antiviral agent against SARS-CoV-2. Thus, VAL and its optimized analogues may be developed as potential and effective antiviral therapeutic agents to benefit many infected patients in the COVID-19 pandemic.

Conflicts of interest

The authors have declared that there are no conflicts of interest.

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