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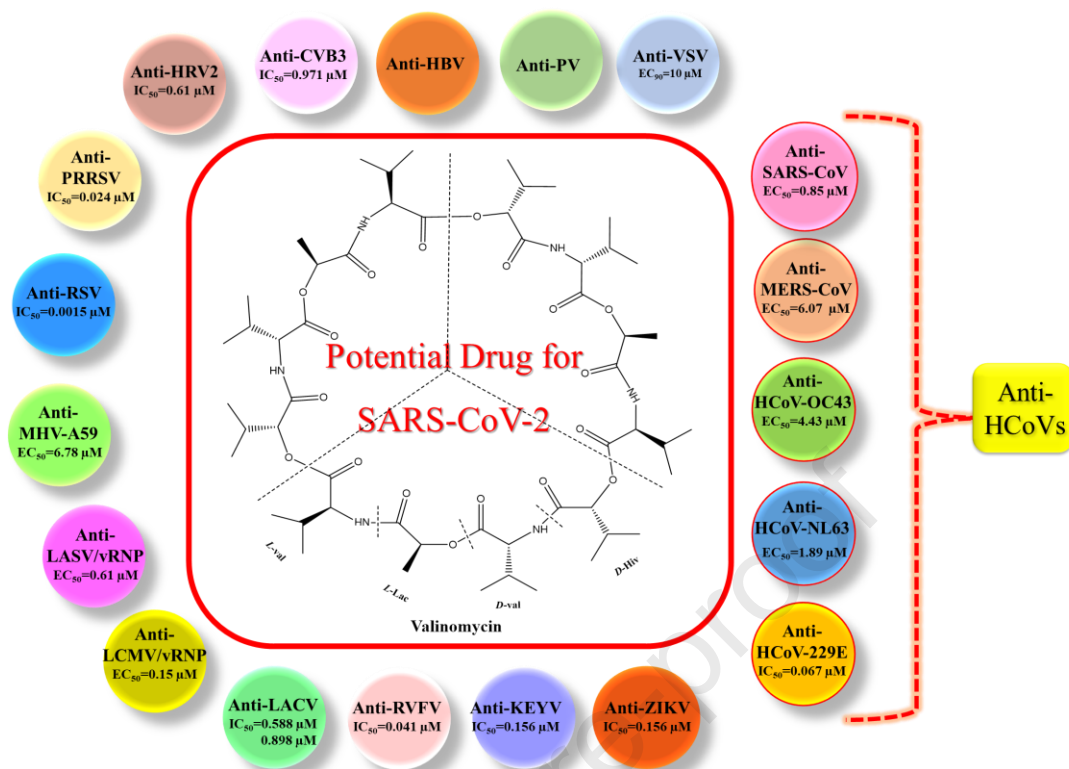
Graphical Abstract

1 **Abstract**

2 Human coronaviruses (HCoVs), including severe acute respiratory syndrome coronavirus

3 (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute

4 respiratory syndrome coronavirus 2 (SARS-CoV-2), have been resulting in global epidemics

5 with heavy morbidity and mortality. Unfortunately, there are currently no specific medicines

6 that can better treat these coronaviruses. Drug repurposing is an effective and economical

7 strategy for drug discovery from existing drugs, natural products, and synthetic compounds.

8 In this review, the broad-spectrum antiviral activity of valinomycin (VAL), especially its

9 activity against coronaviruses such as SARS-CoV, MERS-CoV, human coronavirus OC43

10 (HCoV-OC43), were summarized, it highlights that VAL has tremendous potential for use as

11 a novel antiviral agent against SARS-CoV-2.

12 **Keywords** Coronavirus; SARS-CoV-2; Drug repurposing; Valinomycin; Antiviral agent

13 **1 Introduction**

14 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), provisionally

15 named 2019 novel coronavirus (2019-nCoV), is the causal agent of the outbreak of

16 coronavirus disease 2019 (COVID-19) [1, 2]. Coronaviruses (CoVs) are a group of enveloped

17 viruses with a single-strand, positive-sense RNA genome ranging from approximately 26 to

18 32 kilobase [3]. Its genome organization for CoVs is similar: about two-thirds of the 5'-

19 proximal genome contains the ORF1a/b replica gene, and the remainder encodes the spike,

20 envelope, membrane, nucleocapsid structural proteins, and several accessory proteins [4].

21 Coronaviruses are divided into four genera: alphacoronaviruses (α-CoVs), betacoronaviruses

22 (β-CoV), gammacoronaviruses (γ-CoVs), and deltacoronaviruses (δ-CoVs) [5]. They have the

23 potential to cause respiratory, enteric, hepatic, and neurologic diseases among humans, other

24 mammals, and birds [6, 7]. In detail, only α-CoVs and β-CoVs can infect humans while most

25 γ-CoVs and δ-CoVs can infect avian species. Since the first isolation of the coronavirus in

26 1937, seven coronaviruses were currently known to cause human disease, as shown in Table 1.

27 In the mid-1960, two coronaviruses human coronavirus 229E (HCoV-229E) and human

28 coronavirus OC43 (HCoV-OC43) were isolated from human [8-13]. Subsequently, the other

29 five human coronaviruses (HCoVs) were identified: severe acute respiratory syndrome

30 coronavirus (SARS-CoV) [14-16], human coronavirus NL63 (HCoV-NL63) [17-19], human

31 coronavirus HKU1 (HCoV-HKU1) [20-22], Middle East respiratory syndrome coronavirus

32 (MERS-CoV) [23, 24], and SARS-CoV-2 [1, 2]. HCoV-229E, HCoV-OC43, HCoV-NL63,

33 and HCoV-HKU1 are prevalent and typically cause common cold symptoms in

34 immunocompetent persons. However, SARS-CoV, MERS-CoV, and SARS-CoV-2 can cause

35 severe acute respiratory syndrome and have subsequent person-to-person transmission [25-

36 27]. SARS-CoV was the first coronavirus that caused an epidemic of severe acute respiratory

37 syndrome (SARS), which was associated with an outbreak of atypical pneumonia [28]. The

38 major clinical features include persistent high fever and mild respiratory symptoms but

39 rapidly progressed to pneumonia [29-32]. According to vital statistics data from 2002 to 2003,

40 SARS-CoV resulted in 8437 cases and 813 death in 29 countries [33]. Then, MERS-CoV was

41 the causal agent of Middle East severe respiratory disease outbreaks, which plagued 2494

42 people and caused 858 death between 2012 and 2019 in 27 countries worldwide [34]. Data as

43 of 18 May 2020, SARS-CoV-2 resulted in 4,534,731confirmed cases and 307,537 confirmed

44 deaths in 216 countries, areas, or territories. The news about the new coronavirus disease

45 (COVID-19) outbreak is ongoing updated by the World Health Organization (WHO)

46 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). Though SARS-CoV-2

47 was a new human pathogen that causes severe respiratory illness, it's similar to MERS-CoV

48 and SARS-CoV [1, 35]. Currently, no specific drugs are available to target SARS-CoV-2.

49 Fortunately, drug repurposing has been proved to be an effective and economical drug

50 discovery strategy from existing anti-CoVs drugs [36, 37]. Therefore, the inhibitors with

51 broad-spectrum antiviral activity against HCoVs, especially MERS-CoV and SARS-CoV,

52 may be potent drug candidates against SARS-CoV-2 [38, 39].

53   VAL has broad-spectrum biological activities such as antitumor [40], antibacterial

54 [41], antifungal [42], insecticidal [43], and antiviral activities [44, 45]. It was first isolated

55 from *Streptomyces fulvissimus* by Brockmann et al in 1955 [46]. The structure of VAL was

56 determined as 12 stereogenic centers consisting of a three repeating sequence of the tetramer

57    $_D$-α-hydroxyisovaleric acid-$_D$-valine-$_L$-lactate-$_L$-valine ($_D$-Hiv-$_D$-Val-$_L$-Lac-$_L$-Val). VAL can

58   selectively transport K^+ across model lipid bilayer membranes [47]. Furthermore, VAL is also

59   a respiratory chain ionophore inhibitor that inhibits oxidative phosphorylation by increasing

60   the permeability of the mitochondrial inner membrane to K^+ [48]. Total synthesis of

61   valinomycin has been mentioned in the previous article [42, 49-51]. On the other hand, the

62   gene cluster of VAL has been characterized [44, 52]. The biosynthesis pathway for VAL has

63   been proposed, and as shown in Figure 1 [52, 53]. VAL is biosynthesized by a nonribosomal

64   peptide synthetase (NRPS) called tetramodular valinomycin synthetase (VlmSyn), which is

65   coded by two large NRPS genes with distinctive domain organization A-KR-T-C-A-T-E-C

66   for *vlm1* (10,287 bp) and A-KR-T-C-A-T-TE for *vlm2* (7,968 bp), and functionally defined

67   small ORFs [44, 53, 54]. Moreover, VlmSyn is divided into four modules, which consist of

68   domains with adenylation (A), ketoreductase (KR), thiolation (T), condensation (C),

69   epimerase (E), and thioesterase (TE) functions. Each module assembles $_D$-Hiv, $_D$-Val, $_L$-Lac,

70   or $_L$-Val to form a tetradepsipeptide basic unit. Besides, α-ketoisovalerate (α-Kiv), pyruvate,

71   and $_L$-Val as basic precursors are needed. KR domain reduces α-Kiv to $_D$-Hiv in Module 1. E

72   domain transfers $_L$-Val to $_D$-Val in Module 2. Pyruvate is reduced to $_L$-Lac *via* KR domain in

73   Module 3. Besides, production levels of valinomycin in *Streptomyces* and *Escherichia coli*

74   were summarized, as shown in Table 2 [41, 53-67].

75   Valinomycin (VAL), a cyclodepsipeptide antibiotic acting as a potassium ion

76   transporter, was first considered as the most potent inhibitor of SARS-CoV among more than

77   10,000 drug candidates by Wu, Jan et al. in 2004 [45]. Then, researchers reported potential

78   broad-spectrum activity of VAL against the other four human coronaviruses such as MERS-

79   CoV, HCoV-OC43, HCoV-NL63. Furthermore, VAL showed broadly antiviral activities

80   against other viruses, including one subfamily *Coronavirus* mouse hepatitis virus A59 (MHV-

81   A59), vesicular stomatitis virus (VSV), poliovirus (PV), hepatitis B virus (HBV),

82   enteroviruses (coxsackievirus B3 and human rhinovirus 2), porcine reproductive and

83   respiratory syndrome virus (PRRSV), respiratory syncytial virus (RSV), Lassa virus (LASV),

84 lymphocytic choriomeningitis mammarenavirus (LCMV), La Crosse virus (LACV), Rift

85 Valley fever virus (RVFV), Keystone virus (KEYV), and Zika virus.

86   This review summarizes the broad-spectrum antiviral activities of VAL against

87 nineteen viruses, including HCoV-229E, HCoV-OC43, HCoVNL63, SARS-CoV and MERS-

88 CoV. And the mechanism of VAL on viruses was discussed. Furthermore, it demonstrated

89 that VAL may be developed as an antiviral therapeutic agent with the potential to combat

90 fast-spreading coronavirus disease 2019 (COVID-19).

91 **2 Antiviral activity of valinomycin against human coronaviruses**

92   In this section, we describe the antiviral activity of VAL against five HCoVs,

93 including SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-NL63, HCoV-229E. The antiviral

94 activities of VAL against coronaviruses are shown in Table 3.

95 **2.1 SARS-CoV**

96   SARS-CoV was the causal agent of the severe acute respiratory syndrome (SARS)

97 epidemic, which was caused by a laboratory accident or probably transmitted to humans by

98 animals [15, 16]. SARS-CoV enters its host cell by the Angiotensin-converting enzyme 2

99 (ACE2) receptor, which is a member of the renin-angiotensin system. Besides, its genome

100 organization is similar to that of previously known coronaviruses, for example, HCoV–229E

101 and HCoV–OC43 [14, 16]. The development of drugs against SARS-CoV was significant and

102 necessary. The activity of the valinomycin against SARS-CoV was reported by Wong's group

103 at Academia Sinica, Taiwan [45]. During multiple concentrations, the 50% effective

104 concentrations for the inhibition of viral replication (EC_{50}, 0.85 µM) and host growth (CC_{50},

105 68 µM) were determined by a cell-based assay using SARS virus and Vero E6, and used to

106 evaluate the activity of VAL. The results indicated that VAL exhibited the most potent

107 antiviral activity against SARS-CoV among nearly 10,000 antiviral agents, including existing

108 drugs, natural products, and synthetic compounds. However, the mode of action of VAL

109 against SARS-CoV is not yet known [44].

110   **2.2 MERS-CoV**

111   MERS-CoV, formerly known as a novel coronavirus (NCoV), was identified as the

112   etiological agent of the Middle East respiratory syndrome (MERS), which causes a severe

113   respiratory illness with symptoms of fever, cough, and shortness of breath [23]. Since 2012,

114   the WHO has reported 2494 laboratory-confirmed cases of infection with MERS-CoV, of

115   which 858 MERS-CoV associated deaths have occurred in 27 countries

116   (https://www.who.int/emergencies/mers-cov/en/). In 2019, German scientists found that E3

117   ligase S-phase kinase-associated protein 2 (SKP2) executes lysine-48-linked

118   polyubiquitination of Benclin 1 (BECN1), leading to its proteasomal degradation. SKP2-

119   BECN1 Link is a potent target for host-directed antiviral drugs and other autophagy-sensitive

120   diseases. Notably, VAL is considered to act as an SKP2 inhibitor, which enhances BECN1

121   protein stability and autophagy and efficiently reduces the replication of MERS-CoV [68, 69].

122   VAL inhibited MERS-CoV replication by up to 1000-fold at 48 h post-infection at a

123   concentration of 10 μM, while it enhanced ATG14 oligomerization about 2-fold and

124   increased the number of autolysosomes by >2-fold. [68]. At the same time, National Institute

125   for Viral Disease Control and Prevention, China CDC, Beijing, China, also revealed that VAL

126   inhibited the replication of MERS-CoV with an EC_{50} value of 6.07 μM and CC_{50} value of

127   5.88 μM using an experimental design of dose-response studies *in vitro* [70]. Recently, it

128   demonstrated that VAL exhibited inhibitory activity with an IC_{50} value of 0.005 μM [71].

129   **2.3 HCoV-OC43**

130   HCoV-OC43 is a respiratory epithelial virus, belonging to genus Betacoronavirus,

131   subgenus *Embecovirus* of the family *Coronaviridae*, which widely spread in the population

132   and is related to the common cold [72]. Compared with spike (S) gene sequences of HCoV-

133   OC43 and bovine coronavirus (BCoV), which is another host-type of betacoronavirus that

134   causes respiratory disease, calf diarrhea, and winter dysentery in cattle, it was proposed that

135   bovine-to-human spillover of BCoV leaded to HCoV-OC43 infection [73]. 9-O-acetylated

136   sialic acids are the cellular receptor of HCoV-OC43, as well as HCoV-HKU1 [13]. In 2019,

137   the effect of VAL on HCoV-OC43 was reported by China CDC, Beijing, in 2019 [70]. VAL

138   inhibited the replication of HCoV-OC43 with an EC_{50} value of 4.43 µM and a CC_{50} value of

139   6.15 µM. The mechanism of action VAL against HCoV-OC43 is not yet reported.

140   **2.4 HCoV-NL63**

141   HCoV-NL63 is an enveloped positive-sense single-stranded RNA virus that was first

142   isolated from a seven-month-old child with severe lower respiratory tract in Amsterdam in

143   2004 [17, 18]. Same as SARS-CoV and SARS-CoV-2, HCoV-NL63 enters its host cell by

144   binding to the ACE2 receptor [19]. HCoV-NL63 can cause mild upper respiratory illness with

145   symptoms of cough, fever, or rhinorrhea, or result in more serious lower respiratory tract

146   infections with symptoms of bronchiolitis and croup [74, 75]. Along with HCoV-OC43,

147   HCoV-NL63 are common causes of upper respiratory tract infections, which occur more

148   frequently than HCoV-HKU1 and HCoV-229E infections in early childhood [76-78]. VAL

149   showed inhibitory activity against HCoV-NL63 with an EC_{50} value of 1.68 µM and a CC_{50}

150   value of 4.12 µM [70]. The mechanism of action of VAL against HCoV-NL63 has not been

151   investigated.

152   **2.5 HCoV-229E**

153   HCoV-229E is the first identified human coronavirus that belongs to a member of the

154   genus *Alphacoronavirus* and subgenus *Duvinacovirus* [12]. HCoV-229E and HCoV-OC43

155   are alphacoronaviruses among the seven known human coronaviruses, while the other five

156   human coronaviruses are betacoronavirus. HCoV-229E, along with HCoV-OC43, were

157   considered the cause of the virus that causes the common cold with the symptoms of lower

158   respiratory tract infections and otitis media [76]. The cellular receptor of HCoV-229E is

159   Human Aminopeptidase N (HAPN) [11]. During the rapid antiviral screening to identify

160   potential antivirals against the La Crosse virus, the VAL active against HCoV-229E infection

161   was reported by Sandler et al. [71]. VAL showed inhibitory activity with an IC_{50} value of

162   0.067 µM and exhibited a potent inhibitory against the replication of HCoV-229E at the

163   concentration of 10 µM. The mechanism of VAL against HCoV-NL63 was unclear.

164 **3 Antiviral activity of valinomycin against other viruses**

165 **3.1 Vesicular stomatitis virus**

166   Vesicular stomatitis virus (VSV) is an enveloped negative-sense single-stranded

167 RNA virus that belongs to the genus *Vesiculovirus* of the family *Rhabdoviridae* [79, 80].

168 VSV is the primary cause of vesicular disease outbreaks in livestock. In 1999, the antiviral

169 activity of valinomycin against VSV was determined [41]. Specific infectivities of partially

170 purified ^{35}S-methionine-labeled VSV had no apparent changes in the presence and absence of

171 valinomycin. The reduction in VSV titer in the presence of VAL may be due to a reduction in

172 the production of virus particles, in comparison with the releasing of noninfectious particles.

173 VAL affected the processing of glycoprotein (G protein). In the presence of VAL, G protein

174 oligosaccharides were sensitive to endo-b-N-acetylglucosaminidase H, which can cleave

175 high-mannose oligosaccharides but not complete processing of complex oligosaccharides. As

176 a result, most of the oligosaccharides in G protein were not converted into VSV G protein

177 with mature structure and function required for transport of G protein to the cell surface and

178 its further incorporation into budding particles. The addition of 10 µM valinomycin to the

179 infected Vero cells within the first three hours, resulted in a 90% reduction in viral titer 12

180 hours after infection. Of course, higher concentrations of valinomycin resulted in an even

181 greater reduction in viral titer.

182 **3.2 Poliovirus**

183   Poliovirus (PV) is a non-enveloped single-stranded positive-sense RNA virus that

184 belongs to the species *Enterovirus C* of family *Picornaviridae*. There are three wild types of

185 poliovirus (PV)–PV-1, PV-2, and PV-3. PV spreads from person to person and can cause

186 devastating epidemics of poliomyelitis. Though two vaccines were used to protect against

187 poliovirus infection, the infectious entry route of PV is still unclear.

188 (https://www.cdc.gov/cpr/polioviruscontainment/diseaseandvirus.htm). According to Irurzun

189 et al. [81], although poliovirus does not require an intact pH to enter into the susceptible cell,

190 its RNA requires an intact concentration of K^+ inside the cells to be uncoated and to enter the

191 cytoplasm. The productive poliovirus entry of poliovirus is blocked by and concanamycin A.

192   In other words, VAL exerted inhibitory effect at the stage of poliovirus replication [82]. The

193   replication of poliovirus was powerfully inhibited by the combination of a vacuolar proton-

194   ATPase inhibitor concanamycin and ionophore antibiotics VAL at the concentration of 80 nM

195   and 50 µM, respectively [81].

196   **3.3 Hepatitis B virus**

197   Hepatitis B virus (HBV) is an enveloped partially double-stranded DNA virus that

198   belongs to the genus *Orthohepadnavirus* of the family *Hepadnaviridae*. HBV was identified

199   as the causative agent of the disease hepatitis B, which is a major cause of global health

200   problems. It is a viral infection that can cause both acute and chronic infection of the liver.

201   Millions of people are infected with chronic hepatitis B annually and led to approximately

202   900,000 deaths in 2005 (https://www.who.int/news-room/fact-sheets/detail/hepatitis-b). The

203   interaction of hepatitis B virus (HBV) polymerase (Pol) with Ca^{2+}-modulated protein

204   S100A10 (p11) is important for performing multiple functions required for viral replication.

205   The activity of VAL against HBV was evaluated through exploring effects of Ca^{2+} on the

206   nuclear localization of HBV Pol and p11 in HepG2 cells, which were incubated within 30 µM

207   VAL for 24 hours. The results showed that VAL promoted Ca^{2+} influx, resulted in an

208   inhibition of the association of HBV Pol–p11 with the promyelocytic leukemia protein PML.

209   In other words, VAL showed anti-HBV activity in viral replication and transcription [83].

210   **3.4 Enteroviruses**

211   Enterovirus is a genus of a non-enveloped single-stranded and positive-sense RNA virus that

212   belongs to the members of the *Picornavirus* family. More than 90 subtypes of enteroviruses

213   have been identified, including rhinovirus and Coxsackievirus B3 (CVB3). CVB3 is one of

214   the major pathogens that may cause hand, foot, and mouth disease (HFMD), as well as

215   disease of muscles, lungs, and heart in infants and young children. Human rhinovirus (HRV)

216   is the most common viral infectious agent in humans and is the major cause of the common

217   cold, which causes billions of dollars losses annually in higher medical costs and lost

218   productivity at work [84]. Berka et al. reported that HRV2 type 2 (HRV2) uncoating and

219   RNA translocation are not affected by a pH gradient, but are affected by membrane potential

220 between the acidic endosome lumen and the neutral cytoplasm [85]. VAL depolarized the

221 host membrane, thereby preventing the fusion of the HRV2 with the endosomal membranes.

222 Besides, VAL had a slight inhibitory effect on the synthesis of cellular protein, and the results

223 were only shown when compared with other drugs [82, 85]. Sandler et al. demonstrated that

224 VAL exhibited inhibitory activity against CVB3 and HRV2 during culture tests on Huh7 cells

225 with IC_{50} values of 0.971 μM and 0.61 μM, respectively [71].

226 **3.5 Porcine reproductive and respiratory syndrome virus**

227 Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped

228 positive-sense single-strand RNA virus that causes porcine reproductive and respiratory

229 syndrome (PRRSV). It belongs to the genus *Arterivirus* of the family *Arteriviridae*. Since its

230 first emergence in the USA in 1987 and Europe in 1990, PRRSV causes late-term

231 reproductive failure in breeding severe stock pneumonia in neonatal pigs in swine worldwide

232 (https://www.prrs.com/en/prrs/). Karuppannan et al. established a high-throughput screening

233 method to screen potent agents inhibiting the replication of PRRSV in a library of 502

234 purified natural compounds. VAL is known to act on ion channels of cells membrane. It did

235 not block the binding and entry steps of the PRRSV but took action during the subsequent

236 virus replication process [86]. VAL was described as one of the most potent specific

237 inhibitors of the PRRSV replication with $IC_{50}= 24$ nM in infected MARC-145 cells.

238 **3.6 Respiratory syncytial virus**

239 Respiratory syncytial virus (RSV) is an enveloped negative-sense single-stranded

240 RNA   virus   that   usually   causes   mild   and   cold-like   symptoms

241 (https://www.cdc.gov/rsv/index.html). RSV is a leading cause of lower respiratory tract

242 infections in infancy and childhood worldwide. Though ribavirin is used for clinical therapy,

243 there is no effective vaccine against RSV. According to Norris et al. [82], the cardiac

244 glycosides were identified as inhibitors of the membrane-bound Na^+/K^+-ATPase against the

245 replication of RSV. Notably, VAL has a high selectivity for intracellular K^+ relative to Na^+

246 [47]. This mechanism of action of VAL on RSV at this stage of the replication cycle is the

247 same as that of poliovirus. Therefore, VAL exerted antiviral activity at a post-entry stage,

248 mainly affecting the viral transcription and replication stages of the viral life cycle [81, 82].

249 Therefore, the VAL was screened out as an anti-RSV inhibitor in human epithelial type 2

250 cells and primary nasal epithelial cells RSV with an IC_{50} value of 0.0015 µM and a CC_{50}

251 value of 2.705 µM [82].

3.7 Mouse hepatitis virus A59

253 Mouse hepatitis virus A59 (MHV-A59) is an enveloped positive-sense single-

254 stranded RNA virus that belongs to a member of the genus *Betacoronavirus* within the

255 subfamily *Coronavirinae*. It caused a variety of syndromes in susceptible strains of mice,

256 including hepatitis, thymus involution, and hypergammaglobulinaemia [87]. It was reported

257 that the genomes of the MHV-A59 display 71% identity with two HCoV-OC43 variants,

258 which were obtained from the American Type Culture Collection (ATCC) and a clinical

259 isolate [88]. The cellular receptor of MHV-A59 is Carcinoembryonic antigen-related cell

260 adhesion molecule 1 (biliary glycoprotein) (CEACAM1) [89]. Later, China CDC et al.

261 reported that VAL inhibited the replication of MHV-A59 at the concentration of EC50=6.78

262 µM and CC_{50}=5.11 µM [70]. However, the researchers have not further studied the

263 mechanism of VAL against MHV-A59.

3.8 Lassa virus and lymphocytic choriomeningitis virus

265 Lassa virus (LASV) is an enveloped single-stranded segmented ambisense RNA virus

266 that causes Lassa hemorrhagic fever, which is a highly prevalent febrile disease associated

267 with high morbidity and significant mortality in West Africa

268 (https://www.cdc.gov/vhf/lassa/). Lymphocytic choriomeningitis mammarenavirus (LCMV)

269 was the initially isolated *arenavirus* that belongs to a member of the family *Arenaviridae* in

270 1933. LCMV was identified as the causative agent of lymphocytic choriomeningitis (LCM),

271 which is associated with aseptic meningitis, encephalitis, or meningoencephalitis

272 (https://www.cdc.gov/vhf/lcm/index.html). Virus ribonucleoprotein (vRNP) is responsible for

273 directing viral RNA genome replication and gene transcription of LASV and LCMV. Cubitt

274 et al. established a high throughput screen to identify VAL, which was an effective inhibitor

275 of the activity of LASV/vRNP and LCMV/vRNP in the cell-based, infectious-free, platform.

276   VAL exhibited strong inhibitory effect on LCMV and LASV vRNP activity with $EC_{50}=0.61$

277   µM ($CC_{50}=1.22$ µM), and $EC_{50}=0.15$ µM ($CC_{50}=1.93$ µM), respectively [90].

278   **3.9 Bunyaviruses**

279   Bunyaviruses are spherical or pleomorphic, enveloped viruses that infect humans and

280   cause rashes and fever and even encephalitis. Bunyaviruses belong to the family *Bunyaviridae*,

281   including La Crosse virus (LACV), Rift Valley fever virus (RVFV), Keystone virus (KEYV),

282   and so on. These viruses are transmitted to humans through bites or contact with the blood or

283   tissues of infected animals. LACV is a California serogroup bunyavirus that can cause

284   encephalitis or inflammation of the brain, but no specific drugs target the LACV. RVFV is an

285   enveloped negative single-stranded RNA virus that causes Rift Valley fever (RVF), which is

286   a viral zoonosis that affects animals and can infect humans. KEYV is a mosquito-borne virus

287   that can cause humans with minor symptoms of a rash and fever. In other words,

288   Bunyaviruses can infect humans and cause rashes and fever and even encephalitis. Sandler et

289   al. established a rapid antiviral screening method to screen several potential antiviral

290   molecules, including known and novel antivirals, which exhibited antiviral activity against

291   LACV [71]. According to Mankouri et al. [91] , it demonstrated that the activity of cellular

292   K^+ channels is necessary to cause productive infection of several bunyaviruses, and K^+

293   channels as targets to impede virus entry, infection, and disease. Besides, Sandler et al.

294   reported that VAL does not reduce viral particle infectivity and the ability of virus binding to

295   host cells, but may preclude virus replication by altering cellular K^+ gradient. In other words,

296   VAL exerted antiviral activity at the entry stages of bunyaviruses infection within host cells

297   [71]. VAL potently inhibited LACV replication with an IC_{50} value of 0.588 µM and 0.898

298   µM during culture tests on Huh7 cells and Vero-E6 cells, respectively. Moreover, Treatment

299   with the VAL resulted in a viral titer reduction in the replication of RVFV and KEYV with

300   IC_{50} values of 0.041 µM and 0.156 µM, respectively [71].

301   **3.10 Zika virus**

302   Zika virus (ZIKV) is an enveloped positive-sense single-stranded RNA virus that

303   belongs to the genus *Flavivirus of* the family *Flaviviridae*. ZIKV was first identified from

304 monkeys in 1947 and from humans in 1952. ZIKV is mainly transmitted by the bite of

305 mosquitoes and causes an outbreak of mild symptoms, which is similar to a very mild form of

306 dengue fever (https://www.who.int/news-room/fact-sheets/detail/zika-virus). Unfortunately,

307 there is no specific medicine and vaccines for ZIKV infection. Recently, the outbreak of

308 ZIKV disease has been reported evidence of mosquito-transmitted Zika infection in a total of

309 86 countries and territories. Although there is no specific report on the mechanism of VAL on

310 ZIKV, Sandler et al. also mentioned that potassium ionophore VAL precluded the ZIKV

311 infection by altering cellular K^+ gradient, which is a conserved and vital host factor in virus

312 replication. ZIKV was sensitive to VAL, and ZIKV infection was restricted completely with

313 above 0.5 μM VAL treatment. Besides, VAL had activity against ZIKV with an IC_{50} value of

314 0.078 μM. [71].

4 Conclusion and prospect

316 The studies have shown that LD50 of VAL given i.p. in mice was 1.7 mg/kg, LD50

317 for liposome incorporated valinomycin (MVL-VM) was more than 50 mg/kg. LD50 of VAL

318 form given i.v. is shown to be 0.18 mg/kg, where the LD50 for MLV-VM passed through a

319 0.6-μm filter was greater than 10 mg/kg [92]. Likewise, the LD50 of VAL is shown to be 0.98

320 and 4.14 mg/kg, when given to mice by intraperitoneal and subcutaneous injection,

321 respectively [93]. However, the cytotoxicity test using Vero E6 cells indicated that VAL is

322 non-inhibitory to Vero E6 at concentrations higher than four times the anti-SARS

323 concentrations [45]. Furthermore, Sandler et al. discovered that no significant cellular toxicity

324 was observed either by measuring gross cellular morphology or cellular ATP levels when the

325 concentration of VAL was below 10 μM. The effective doses of VAL against the replication

326 of viruses are often less than 10 μM (Table 3). Namely, VAL has antiviral effects in a variety

327 of cell types at non-cytotoxic doses and reduces virus titers and cell-associated virus genomes

328 [71]. Despite VAL is not an FDA-approved antiviral drug for human, modification of the

329 structure of VAL may reduce drug's toxicity while maintaining antiviral activity *in vivo*. In

330 other words, it is necessary to further study the antiviral activity and drug's safety of

331 synthesized derivatives and analogs of VAL in animal models or human clinical trials [94, 95].

332 On January 30, 2020, WHO declared the outbreak of COVID-19 a Public Health

333 Emergency of International Concern (PHEIC) and made a series of temporary

334 recommendations. Due to the lack of antiviral therapies and vaccines, the main treatment

335 strategy for COVID-19 is supportive care, supplemented by broad-spectrum antibiotics,

336 antivirals, corticosteroids, nucleoside analogues, protease inhibitors, and recovery plasma,

337 and so on [96]. Recently, it demonstrated that remdesivir and chloroquine exhibited potential

338 antiviral activity against SARS-CoV-2 during culture tests on Vero E6 cells with 50%

339 effective concentrations of 0.77 μM and 1.13 μM, respectively [97]. Of note, the genome of

340 SARS-CoV-2 shares 79.6% and 50% sequence identity to SARS-CoV and MERS-CoV,

341 respectively, and is 96% identical at the whole-genome level to a bat coronavirus [2, 35].

342 VAL was remained as the most potent inhibitor for the replication of SARS-CoV with a value

343 of $EC_{50}=0.85$ μM and $CC_{50}=68$ μM using a cell-based assay.

344 Furthermore, this review summaries a list of nineteen viruses with corresponding

345 EC50 and IC50 to VAL, and five of these viruses were HCoVs, including HCoV-229E,

346 HCoV-OC43, HCoVNL63, SARS-CoV, and MERS-CoV. Although the mechanism of VAL

347 against certain viruses has not been reported, such as SARS-CoV, HCoV-OC43, and HCoV-

348 NL63, its mechanism on some specific viruses has been described in the relevant summary.

349 VAL exhibited antiviral activity against non-enveloped viruses such as poliovirus and HRV2.

350 Studies on the HRV2 have shown that VAL depolarized the host membrane to block viral

351 fusion with the endosomal membranes and had a slight inhibitory effect on the synthesis of

352 cellular protein. VAL showed an inhibitory effect at the replication phase of the poliovirus

353 life cycle. It is worth noting that this mechanism of VAL on the non-enveloped virus

354 poliovirus is the same as that against enveloped virus RSV. Also, VAL has shown other

355 mechanisms against the replication of enveloped viruses. For instance, VAL act as an

356 inhibitor SKP2 that enhances autophagy effectively and reduces the replication of MERS-

357 CoV. VAL inhibited the replication of VSV by affected the processing of G protein.

358 Specifically, in the presence of VAL, most of the oligosaccharides in VSV G proteins were

359   not converted into structurally and functionally mature form, which is required for transport

360   of G protein to the cell surface and its further incorporation into budding particles. VAL also

361   inhibited the activity of LCMV and LASV vRNP, which are responsible for directing viral

362   RNA genome replication and gene transcription. In addition, the ionophore antibiotic VAL

363   disrupted the K^+ gradient that is a conserved and vital host factor in virus replication, leading

364   to abnormal cellular events, including endocytosis required for efficient virus entry. These

365   findings indicate that VAL may be repurposed as an antiviral agent against SARS-CoV-2.

366   Thus, VAL and its optimized analogues may be developed as potential and effective antiviral

367   therapeutic agents to benefit many infected patients in the COVID-19 pandemic.

368

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599

600   **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]

601   **Abbreviations** HCoV: Human coronavirus; SARS-CoV: Severe acute respiratory syndrome

602   coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; SARS-CoV-2: Severe acute

603   respiratory syndrome coronavirus 2; α-CoV: alphacoronaviruses; β-CoV: betacoronaviruses; HAPN:

604   Human Aminopeptidase N; ACE2: Angiotensin-converting enzyme 2; DPP4: Dipeptidyl peptidase 4.

605

606 **Table 2** Production levels of valinomycin

Strains	Yield (mg·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]

607

608

609 **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]

610 **Abbreviations** EC_{50}/ EC_{90}: 50% / 90% effective concentration; IC50: 50% inhibitory concentration;

611 VSV: Vesicular stomatitis virus; PRRSV: Porcine reproductive and respiratory syndrome virus; CVB3:

612 Coxsackievirus B3; HRV2: Human rhinovirus 2; RSV: Respiratory syncytial virus; MHV-A59: Mouse

613 hepatitis virus A59; LACV: La Crosse virus; LASV: Lassa virus; LCMV: Lymphocytic

614 choriomeningitis mammarenavirus; vRNP: Virus ribonucleoprotein. LACV: La Crosse virus; RVFV:

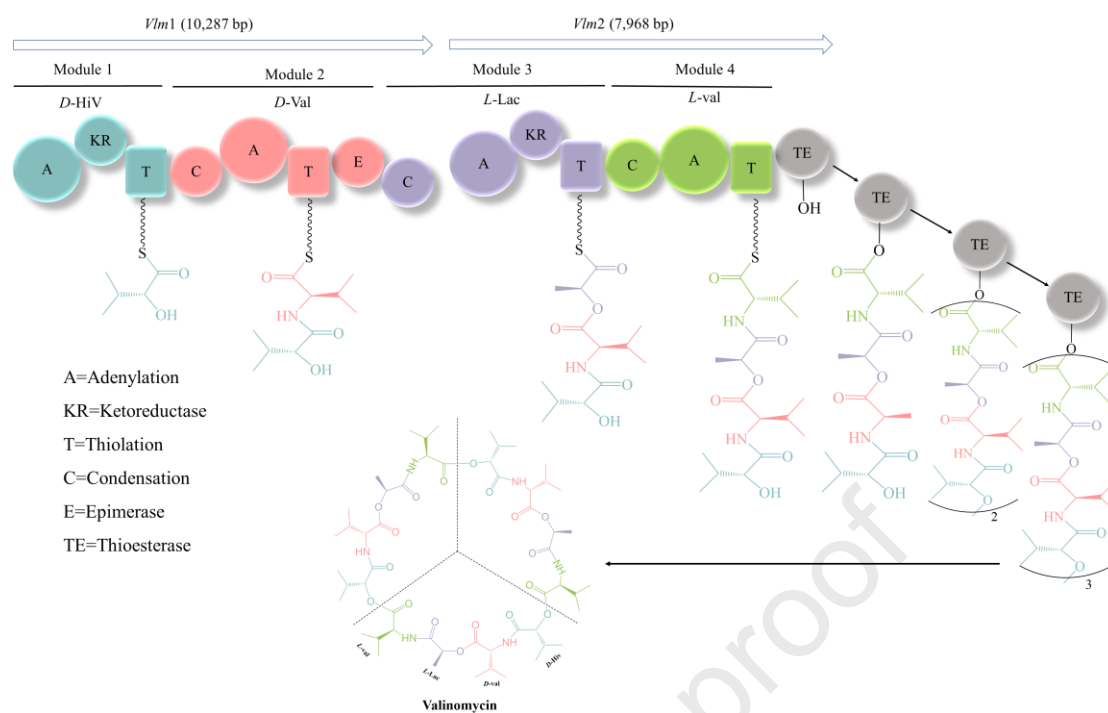615 Rift Valley fever virus; KEYV: Keystone virus; ZIKV: Zika virus.

Figure 1. Proposed pathway for the biosynthesis of valinomycin.

- There are currently no specific medicines to treat COVID-19.

- Drug repurposing is an effective strategy for drug discovery form identified compounds.

- Insight into the antiviral activity and mechanism of valinomycin.

- Valinomycin as a potential antiviral agent against SARS-CoV-2.