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Review

# Broad Spectrum Antiviral Agent Niclosamide and Its Therapeutic Potential

Jimin Xu, Pei-Yong Shi, Hongmin Li, and Jia Zhou*

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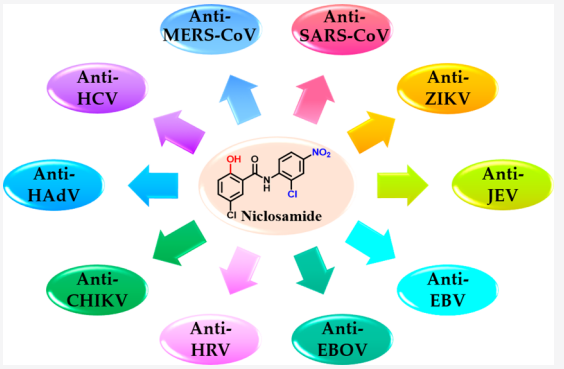
**ABSTRACT:** The recent outbreak of coronavirus disease 2019 (COVID-19) highlights an urgent need for therapeutics. Through a series of drug repurposing screening campaigns, niclosamide, an FDA-approved anthelminthic drug, was found to be effective against various viral infections with nanomolar to micromolar potency such as SARS-CoV, MERS-CoV, ZIKV, HCV, and human adenovirus, indicating its potential as an antiviral agent. In this brief review, we summarize the broad antiviral activity of niclosamide and highlight its potential clinical use in the treatment of COVID-19.




**KEYWORDS:** *niclosamide, broad antiviral agents, coronavirus, SARS-CoV, MERS-CoV, SARS-CoV-2 (COVID-19), flavivirus, Zika virus, Ebola virus, human adenovirus*

The recent outbreak of coronavirus disease 2019 (COVID-19) first detected in Wuhan, China, was caused by a novel betacoronavirus, which was named SARS-CoV-2 (a.k.a. 2019-nCoV) by the International Committee on Taxonomy of Viruses.[1] Coronaviruses (CoVs) are enveloped and positive-sense single-stranded RNA viruses belonging to the family *Coronaviridae* within the order *Nidovirales*. Many coronaviruses infect humans and other mammalian hosts. Coronavirus can be divided into four genera (alpha, beta, gamma, and delta), of which alpha and beta coronaviruses are known to infect humans.[2] Human coronavirus infections are typically mild and rarely associated with severe diseases. However, the epidemics of Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) caused alarming morbidity and mortality.[3] While coronaviruses are often zoonotic, person-to-person transmission has been confirmed for SARS-CoV-2, similar to MERS-CoV and SARS-CoV.[4] As of March 5, 2020, there are more than 95 333 confirmed cases of COVID-19 and at least 3282 reported deaths, indicating that it is a severe public health threat.[5] There is no clinically approved antiviral for coronavirus therapy.[6] Although remdesivir, an experimental antiviral drug candidate by Gilead currently advanced into human clinical trials to treat COVID-19 in both China and the U.S., was reported to improve patient outcomes in a recent study,[7] it is still critical and urgent to search for other effective inhibitors for the potential treatment of COVID-19.

Drug repurposing screens have emerged as an attractive strategy to accelerate new drug discovery and development. This strategy offers various advantages over *de novo* drug discovery featured with key benefits including reduced time, cost, and risk as well as the unique means for safer and more effective drugs to be accessed by patients.[8,9] Niclosamide is an FDA-approved anthelminthic drug that has been widely used in humans to treat tapeworm infections for several decades and is currently listed on the World Health Organization's list of essential medicines.[10,11] Niclosamide exerts its anticestodal effect by inhibiting oxidative phosphorylation and stimulating adenosine triphosphatase activity in the mitochondria.[12] Over the past several years, niclosamide has been identified as a multifunctional drug via drug repurposing screens. It can regulate multiple signaling pathways and biological processes including Wnt/$\beta$-catenin, mTORC1, STAT3, NF-$\kappa$B, Notch, NS2B-NS3 interaction, and pH,[13,14] indicating its potential to treat other human conditions[15] such as cancer,[16−18] bacterial and viral infections,[19−22] and metabolic diseases.[23] These broad biological activities of niclosamide including relevant cell

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signaling pathways were briefly reviewed by Chen et al.[15] In this short review, we focus on summarizing the broad antiviral activities of niclosamide (Figure 1) and highlighting its therapeutic potential in combating COVID-19.

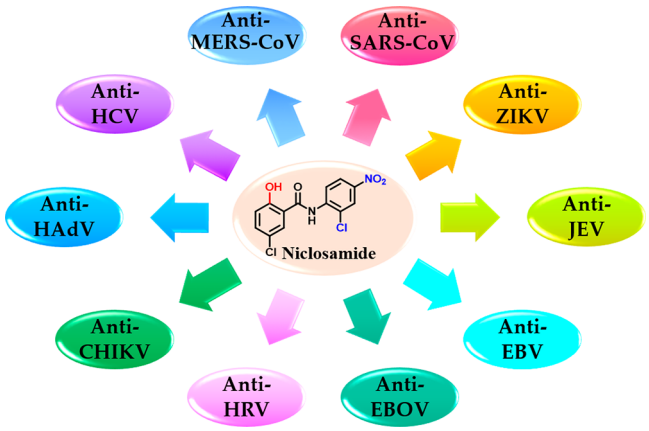


**Figure 1.** Niclosamide has great potential in being repurposed to treat a variety of viral infections, such as severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), Zika virus (ZIKV), Japanese encephalitis virus (JEV), hepatitis C virus (HCV), Ebola virus (EBOV), human rhinoviruses (HRVs), Chikungunya virus (CHIKV), human adenovirus (HAdV), and Epstein−Barr virus (EBV). We envision that this broad spectrum of antival activities may offer the therapeutic potential to be extended to combat fast-spreading coronavirus disease 2019 (COVID-19), given its inexpensive and low *in vivo* toxicity profile as an FDA-approved drug in clinical use.

## NICLOSAMIDE AND VIRAL INFECTIONS

**Niclosamide and Coronavirus.** Coronaviruses are a group of enveloped and nonsegmented positive-sense RNA viruses with very large genome size ranging from approximately 27 to 34 kb. Infections with human strains HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 usually cause mild, self-limiting respiratory infections such as the common cold.[2,24] Nevertheless, in the past 17 years, three beta coronaviruses (SARS-CoV, MERS-CoV, and this year's SARS-CoV-2) have caused severe human disease pandemics associated with high morbidity and mortality. The outbreak of SARS in southern China between November 2002 and July 2003 eventually resulted in 8098 confirmed cases and 774 deaths reported in 17 countries with a mortality rate of 9%, while MERS, first identified in Saudi Arabia in 2012, has caused a total of 2519 laboratory-confirmed cases including 866 associated deaths with a fatality rate of nearly 34% at the end of January 2020.[25,26] The lack of effective treatment for coronavirus infections poses a great challenge to clinical management and highlights the urgent need for new drug discovery. Wu et al. found that niclosamide was able to inhibit SARS-CoV replication and totally abolished viral antigen synthesis at a concentration of 1.56 $\mu$M after screening a small marketed drug library.[27] Niclosamide suppressed the cytopathic effect (CPE) of SARS-CoV at a concentration of as low as 1 $\mu$M and inhibited SARS-CoV replication with an $EC_{50}$ value of less than 0.1 $\mu$M in Vero E6 cells.[28] SARS-CoV 3CL protease plays an important role in replicase polyprotein processing and serves as a key target for anti-SARS drug discovery.[29−31] A series of 2-chloro-4-nitroanilide derivatives was discovered as potent inhibitors against SARS-CoV 3CL protease. Interestingly, niclosamide showed no obvious inhibitory activity against SARS-CoV 3CL protease up to 50 $\mu$M, and mechanistically, it may exert its anti-SARS activity via other modes of action.[32]

Gassen et al. revealed that E3 ligase S-phase kinase-associated protein 2 (SKP2) executes lysine-48-linked polyubiquitination of Benclin 1 (BECN1), resulting in its proteasomal degradation. SKP2 inhibition increases the BENC1 level, enhances autophagy, and efficiently reduces MERS-CoV replication.[33] Niclosamide was reported to inhibit MERS-CoV replication by up to 1000-fold at 48 h p.i. at a concentration of 10 $\mu$M, while it enhanced the BENC1 level and ATG14 oligomerization, increased the number of autolysosomes by >2-fold, and affected the autophagic flux in the MERS-CoV-infected cells.[33] Since niclosamide is a multifunctional drug, we cannot exclude the possibility that it exerts its anti-MERS activity by regulating other targets besides SKP2 inhibition.

**Niclosamide and Flavivirus.** Flavivirus, a genus of viruses in the family *Flaviviridae*, includes the Zika virus (ZIKV), dengue virus four serotypes (DENV 1−4), West Nile virus (WNV), yellow fever virus (YFV), and Japanese encephalitis virus (JEV). Many of these viruses are significant human pathogens. Among these viruses, ZIKV is a mosquito-borne flavivirus that is transmitted primarily by *Aedes* mosquitoes. ZIKV infection can cause infants to be born with microcephaly and can trigger neurologic conditions in adults such as Guillain−Barré syndrome, neuropathy, and myelitis.[34−38] Outbreaks of ZIKV infection have been recorded several times (2015 in Brazil, the latest one), and the World Health Organization (WHO) declared ZIKV to be a global public health emergency. Xu et al. used caspase-3 activity as the primary screening assay and discovered niclosamide as a potent inhibitor of ZIKV infection, displaying an $IC_{50}$ value of 0.37 $\mu$M against the intracellular ZIKV RNA level.[22] The time-of-addition studies indicated that niclosamide inhibits ZIKV infection at a postentry stage, probably in a viral RNA replication step. Our research team also identified niclosamide as a potent anti-ZIKV inhibitor through an independent quantitative high-throughput screening (qHTS) campaign and found that niclosamide directly inhibits flavivirus NS2B-NS3 interactions.[14] Protease complex NS2B-NS3 is essential for flaviviral polyprotein processing.[39−41] Our team also found that niclosamide is a broad-spectrum inhibitor against other flaviviruses including DENV-2, WNV, JEV, and YFV, with potencies similar to that for ZIKV.[14]

In addition, Fang et al. developed a CPE-based HTS assay to screen 1280 pharmacologically active compounds and identified niclosamide as a potent JEV inhibitor with micromolar potency.[42] The time-of-addition studies showed that niclosamide inhibits JEV at the stage of replication.

**Niclosamide and Hepatitis C Virus.** Hepatitis C virus (HCV) is an enveloped positive-sense single-strand RNA virus of the family *Flaviviridae* which is transmitted mainly through blood infection. HCV can cause both acute and chronic hepatitis, and hepatitis C is a major cause of liver cancer. It was estimated that about 71 million people have chronic HCV infections worldwide.[43] At present, there is no effective vaccine against hepatitis C, although clinically approved therapeutics are available. Niclosamide was reported to show very promising activity against HCV replication with an $EC_{50}$ value of 0.16 $\mu$M.[44] It likely inhibits HCV replication via


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modulation of the host cell process similar to that of its derivatives nitazoxanide and tizoxanide.[45−47] However, chronic HCV infection requires long-term (several months) antiviral treatment, which may make a host-targeted approach less attractive.

**Niclosamide and Ebola Virus.** Ebola virus (EBOV) is an enveloped negative-sense single-stranded RNA virus that belongs to the genus *Ebolavirus* of the family *Filoviridae*. EBOV is introduced into humans from wild animals and spreads in the human population through person-to-person transmission. Ebola virus disease (EVD), known as Ebola hemorrhagic fever, has a high fatality rate, ranging from 25 to 90% in past outbreaks. Through a systematic screen of FDA-approved drugs, niclosamide was identified as one of the most potent EBOV inhibitors with an $EC_{50}$ value of 1.5 $\mu$M, although its *in vivo* efficacy has not yet been evaluated in animal models.[48]

**Niclosamide and Human Rhinovirus.** Human rhinoviruses (HRVs) are nonenveloped, positive-sense single-stranded RNA viruses that belong to the genus *Enterovirus* of the family *Picornaviridae*. There are more than 100 different HRV strains classified into three species (HRV A−C). HRVs are widespread among humans and the primary cause of the common cold, posing serious health risks for patients with asthma, chronic pulmonary disease, and severe bronchiolitis in infants and children.[49] Niclosamide is a weak lipophilic acid and was reported to inhibit pH-dependent HRV infection with low micromolar $IC_{50}$ values; it suppresses HRV entry by blocking the acidification of the endolysosomal compartments, acting as a proton carrier.[50]

**Niclosamide and Chikungunya Virus.** Chikungunya virus (CHIKV) is a positive-sense single-stranded RNA virus belonging to the genus *Alphavirus* of the family *Togaviridae*. CHIKV causes fever and joint pain, is transmitted by infected female mosquitoes, and is cataloged as a risk group-3 pathogen. Currently, there is no effective antiviral therapy approved for Chikungunya. Niclosamide was discovered as a potent anti-CHIKV inhibitor with a low micromolar $EC_{50}$ value; it not only affects CHIKV entry via blocking low-pH-dependent virus fusion but also inhibits the cell-to-cell transmission of CHIKV infection.[51]

**Niclosamide and Human Adenovirus.** Human adenoviruses (HAdVs) are nonenveloped double-stranded DNA viruses with icosahedral capsids. HAdVs comprise more than 70 different serotypes classified into seven species (HAdV A−G). HAdV infections can cause severe and often life-threatening diseases in immunosuppressed patients. Currently, no specific antiviral therapy is available to treat these infections. Three salicylanilide anthelmintic drugs including niclosamide were screened out as potent anti-HAdV inhibitors. Niclosamide showed very promising anti-HAdV activity with an $EC_{50}$ value of 0.6 $\mu$M in the plaque assay. Subsequent mechanistic studies indicated that niclosamide inhibits the transport of the HAdV particle from the endosome to the nuclear envelop.[52]

**Niclosamide and Epstein−Barr Virus.** Epstein−Barr virus (EBV), also known as human herpesvirus 4, has a toroid-shaped protein core containing a linear double-stranded DNA genome of 184 kb in size which is a member of the gamma subfamily of herpes viruses. EBV is widely spread in humans and infects over 95% of humans in the first decades of their life, resulting in a lymphoproliferative disorder known as infectious mononucleosis. EBV infection was also found to be associated with the development of several types of cancer such as Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma.[53] Huang et al. demonstrated that niclosamide inhibits EBV lytic replication in lymphoma cells and epithelial cells and causes irreversible cell cycle arrest in lytic EBV-infected cells via disrupting mTOR activation, offering the potential to treat acute EBV-associated infectious diseases.[54]

## CONCLUSIONS AND FUTURE DIRECTIONS

Niclosamide has traditionally been used to treat tapeworm infections for many years, and it is inexpensive and well tolerated *in vivo* with an extremely high acute oral $LD_{50}$ value of >5000 mg/kg in rats (niclosamide ethanolamine salt).[10] In human medicines, single oral doses of 0.5, 1, and 2 g of niclosamide are recommended for children under 2 years, children between 2 and 6 years, and children older than 6 years and adults, respectively, to treat infections with *Taenia solium*, *T. saginata*, and *Diphyllobothrium latum*.[10] Human infections with rat tapeworm *Hymenolepis diminuta* were eliminated by 5−7 daily doses of 2 g of niclosamide each, while the treatment of *Hymenolepis nana* infection requires one or several 5−7 day courses of niclosamide treatment. One 7 day course regimen for adults is 2 g of niclosamide on day 1 followed by 1 g daily for 6 days.[10,55] When treating human volunteers each with a single oral dose of 2000 mg of niclosamide, the maximum serum concentration of niclosamide was equivalent to 0.25−6.0 $\mu$g/mL (0.76−18.3 $\mu$M). The wide concentration range was caused by the intraindividual absorption differences. Niclosamide is only partially absorbed from the intestinal tract, and the absorbed part is rapidly eliminated by the kidneys with no cumulative toxic effects in human.[10] Through a series of drug repurposing screening campaigns, niclosamide was found to be effective against a variety of human conditions such as cancer and viral infections. Currently, there are four ongoing human clinical trials of niclosamide in ulcerative colitis, prostate carcinoma, and colorectal cancer in the ClinicalTrails.gov clinical trials registry.[56] Of note, niclosamide has several weaknesses such as unneglectable cytotoxicity and limited aqueous solubility as well as relatively low absorption and oral bioavailability ($F$ = 10%), which may hamper its extensive clinical development as an antiviral agent.[57] Our group has made substantial efforts in medicinal chemistry based on niclosamide as a lead compound and discovered a series of *O*-alkylamino-tethered derivatives as potent and orally bioavailable anticancer agents with improved aqueous solubility[16] and diversified salicylamide derivatives as potent anti-HAdV inhibitors with increased potency (submicromolar $IC_{50}$s) and significantly decreased cytotoxicity likely by targeting different steps in the HAdV life cycle.[58] The ester derivative prodrug of niclosamide was also reported to increase its systemic drug exposure and extend the duration of exposure.[59] The development of nanobased formulations is another useful strategy for improving the pharmacological and pharmacokinetic properties of niclosamide and maximizing its therapeutic potential for clinical applications.[60−62]

The outbreak of COVID-19 has been declared to be a public health emergency of international concern by the WHO, and the development of effective therapies for fast-spreading fatal COVID-19 is in an urgent need. 3CL protease is a key enzyme that is responsible for proteolytic processing and is indispensable for viral replication and the infection process.[6] Recently, the high-resolution crystal structure of SARS-CoV-2


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3CL protease has been solved by Zihe Rao and Haitao Yang (PDB ID: 6LU7, Figure 2), and this may significantly facilitate

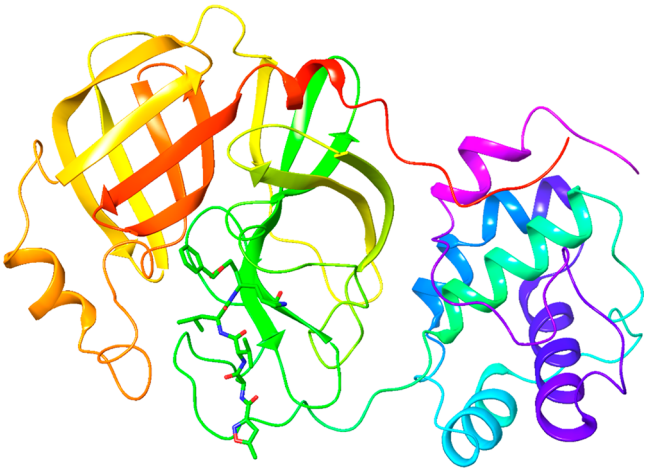

**Figure 2.** Crystal structure of SARS-CoV-2 (2019-nCoV) 3CL protease (PDB ID: 6LU7) recently solved by the team of Zihe Rao and Haitao Yang at ShanghaiTech University, China. The detailed high-resolution crystal structural analysis of the viral main proteinase (Mpro) of SARS-CoV-2 may facilitate the *in silico* screening of existing drugs for drug repurposing or the identification of novel hits from compound libraries by targeting Mpro, which is considered to be a beneficial drug target regulating the activities of the virus replication complex.

the discovery of potent small-molecule inhibitors of COVID-19 by targeting SARS-CoV-2 3CL protease via high-throughput virtual screening of compound libraries or existing drug libraries for drug repurposing. In addition, Wrapp et al. have determined a cryo-EM structure of the SARS-CoV-2 spike (S) glycoprotein trimer in the prefusion conformation that will also facilitate vaccine development and the discovery of antiviral therapeutics for COVID-19.[63] Although these crystal structures may provide new insights and helpful information for future drug discovery, extensive efforts are needed to identify effective binding pockets for small molecules and validate the drug targets.

It is reported that some existing drugs or drug candidates such as remdesivir, an RNA-dependent RNA polymerase (RdRp) inhibitor, and lopinavir/ritonavir (protease inhibitors) against Ebola or HIV may be repurposed through fast-track human clinical trials as effective therapies to combat deadly COVID-19 and save hundreds of patient lives.[7,64,65] Very recently, through screening the existing antiviral drugs, three broad antiviral agents (nitazoxanide, remdesivir, and chloroquine) were found to inhibit SARS-CoV-2 at low micromolar concentrations in Vero E6 cells with $EC_{50}$ values of 2.12, 0.77, and 1.13 $\mu$M, respectively.[66] Nitazoxanide is a prodrug of tizoxanide, which shares considerable structural similarity with niclosamide as a salicylamide derivative.[47] Notably, niclosamide displays promising inhibitory activity against SARS-CoV replication with an $EC_{50}$ value of less than 0.1 $\mu$M in Vero E6 cells and inhibits MERS-CoV replication by up to 1000-fold at 48 h p.i. at a concentration of 10 $\mu$M in Vero B4 cells.[28,33] SARS-CoV-2 belongs to the genus *Betacoronavirus*, the same as SARS-CoV and MERS-CoV, sharing 79.5% sequence identify to that of SARS-CoV.[67] These findings, together with its broad antiviral properties, indicate that niclosamide, an inexpensive and well-tolerated old drug, may be repurposed with therapeutic potential applications to combat COVID-19. We envision that once its anti-SARS-CoV-2 activity is validated in animal models or human clinical trials, niclosamide and its optimized analogues may be developed as effective antiviral therapeutics with the potential to benefit numerous infected patients in this outbreak of COVID-19.

## AUTHOR INFORMATION


### Corresponding Author

**Jia Zhou** − *Chemical Biology Program, Department of Pharmacology and Toxicology and Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, Texas 77555, United States;* orcid.org/0000-0002-2811-1090; Phone: (409) 772-9748; Email: jizhou@utmb.edu

### Authors

**Jimin Xu** − *Chemical Biology Program, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77555, United States*

**Pei-Yong Shi** − *Department of Biochemistry and Molecular Biology and Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, Texas 77555, United States*

**Hongmin Li** − *Wadsworth Center, New York State Department of Health, Albany, New York 12208, United States;* orcid.org/0000-0002-8684-5308

Complete contact information is available at: https://pubs.acs.org/10.1021/acsinfecdis.0c00052


### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS USED

WHO, World Health Organization; COVID-19, coronavirus disease 2019; 2019-nCoV, the 2019 novel coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; HCV, hepatitis C virus; DENV, dengue virus; ZIKV, Zika virus; JEV, Japanese encephalitis virus; WNV, West Nile virus; YFV, yellow fever virus; EBOV, Ebola virus; HRV, human rhinovirus; CHIKV, Chikungunya virus; HAdV, human adenovirus; EBV, Epstein−Barr virus (EBV); SKP2, S-phase kinase-associated protein 2; BECN1, Benclin 1; RdRp, RNA-dependent RNA polymerase; CPE, cytopathic effect; qHTS, quantitative high-throughput screening (qHTS); $EC_{50}$, half maximal effective concentration; $IC_{50}$, half maximal inhibitory concentration; $LD_{50}$, lethal dose, 50%



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