

# Metadichol: an inhibitor of zoonotic viruses; Nipah, Laasa, and rabies

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## Research Article

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**Additional Declarations:** The authors declare potential competing interests as follows: The author is the founder and CEO of Nanorx Inc and is a Major share Holder in the Company.

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# Abstract

Zoonotic viruses, such as coronaviruses, the Ebola virus, the Zika virus, Nipah, Laasa, and rabies, can be transmitted from animals to humans (1). There is a need to develop inhibitors because they can potentially prevent or treat viral infections in humans and animals (2). Metadichol® is a nanoemulsion of long-chain alcohols that is a potent inhibitor of viruses. We present results of in vitro assays showing that it inhibits Laasa, rabies, and Nipah viruses at concentrations of 0.8 to 2.6 µg/ml. It is likely that the binding of metadichol to VDR ( vitamin D receptor) leads to regulation of c-MYC (**MYC Proto-Oncogene, BHLH Transcription Factor**) (which controls expression of SP1(SP1 transcription factor), which is the key step to controlling the viral replication gene GSPT1(**G1 To S Phase Transition 1**). Since metadichol is commercially available and nontoxic, with an LD50 of more than 5000 mg/kg in rats, it could be useful in the treatment of such zoonotic diseases.

## Introduction

Zoonotic viruses can be transmitted from animals to humans and can cause various diseases. They are very common and account for a large proportion of new and emerging infectious diseases in humans (1). Some examples of zoonotic viruses are rabies, Nipah, Lassa, Ebola, and Corona viruses.

The epidemiology of zoonotic viruses depends on the type of virus, the animal reservoir, the mode of transmission, the geographic distribution, and the human risk factors (2). Zoonotic viruses can spread through direct or indirect contact with infected animals or their products, through vectors such as ticks or mosquitoes, or through food or water contamination. Zoonotic viruses can cause outbreaks, epidemics, or pandemics, depending on the level of transmission and the availability of prevention and control measures. Zoonotic viruses pose a major public health challenge and require a One Health approach that integrates human, animal, and environmental health (3–6).

As we have shown previously, Metadichol® inhibits a variety of viruses, including Ebola (7) and Zika (8).

Currently, the epidemiology of Nipah, Lassa, and rabies viruses worldwide is as follows:

**Nipah virus:** The Nipah virus (NiV) is a zoonotic virus that can cause fatal encephalitis in humans. It can be transmitted from animals (such as bats or pigs), contaminated foods, or directly between people. The virus was first recognized in 1999 during an outbreak among pig farmers in Malaysia and Singapore. Since then, it has caused nearly annual outbreaks in Bangladesh and India and sporadic cases in other regions of Asia, the South Pacific, and Australia. The case fatality rate is estimated to be 40–75% ((9). There is no vaccine or specific treatment for Nipah virus infection. However, there are several experimental treatments under development, such as monoclonal antibodies and remdesivir.

There are also efforts to understand the epidemiology, ecology, and pathogenesis of Nipah virus, as well as to develop diagnostic tools and surveillance strategies. (10–13)

**Lassa virus:** The Lassa virus is a zoonotic virus that can cause hemorrhagic fever in humans. It is spread by rodents, mainly in West Africa, where it is endemic in several countries (14). The virus can also be transmitted from person to person through contact with bodily fluids of infected individuals. The case fatality rate is approximately 1%, but it can reach 15% in hospitalized patients. There is no vaccine for Lassa virus infection. The antiviral drug ribavirin (15) can be effective if given early in the course of the disease. There are ongoing clinical trials evaluating new therapeutics and vaccines for Lassa fever, as well as improving diagnostic methods and disease surveillance.

**Rabies virus:** Rabies virus is a zoonotic virus that can cause fatal encephalitis in humans and animals. It is spread by mammals, especially dogs, worldwide. The virus can be transmitted through bites or scratches from infected animals or through exposure to their saliva. The case fatality rate is almost 100% once symptoms appear, but the disease can be prevented by vaccination before or soon after exposure. Approximately 59,000 people die from rabies each year, mostly in Asia and Africa. (16). There is a vaccine for rabies virus infection that can prevent the disease if given before or soon after exposure. However, once symptoms appear, the infection is almost always fatal. Therefore, there is a need to develop more effective postexposure prophylaxis and treatment options. (17) One promising approach is to use recombinant rabies virus harboring the Nipah virus glycoprotein as a dual vaccine against both viruses. (18)

We tested the effects of metadichol on all 3 viruses, and it inhibited all 3 viruses with IC<sub>50</sub>s of 2.24, 2.65 µg/ml, and 0.831 µg/ml against Nipah, rabies, and Lassa viruses, respectively. This process is performed on a newly developed proprietary pseudovirus platform for rapidly screening viral entry inhibitors and neutralizing antibodies. (19).

## Experimental

All the work was outsourced on commercial terms and carried out by Virongy Biosciences (20), Manassas, VA, USA.

Procedure.

Screening of the neutralization activity of metadichol with the HA pseudovirus system. Cytotoxicity assessment in HEK293T cells

Wheongy Biosciences

## Reagent Lots:

HA-RabV: Batch # 111523

HA-LasV: Batch # 041423

HA-NiV: Batch # 091223

Cell Lysis Buffer: Batch # 01022024

D-Luciferin Substrate: Batch #010224

HEK293T Ready-To-Use Cells: Batch #0102024

Firefly Luciferase Assay Buffer Solution: Batch # 111323

Resazurin Cell Viability Assay Kit: Lot # 23R0426

## Procedure:

Metadichol was diluted to achieve assay concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, 1.5625 µg/mL, 0.78125 µg/mL, and 0 µg/mL using the 5 mg/mL stock solution that was shipped to Virongy Biosciences. This was completed through a 1:2 dilution series following the dilution to the working stock concentration.

The assay was run using 15 µL of each metadichol dilution, 15 µL of HEK293T cells (3333 cells/µL) and 45 µL of HA-RabV, HA-LASV, or HA-NiV. The plates were allowed to incubate for 16 hours after infection. After incubation, the cells were lysed with 7.5 µL of 10X lysis buffer and orbital shaking. Then, 25 µL of D-luciferin substrate was added to each well, and the plate was read using the luciferase protocol on Promega Glomax. All pseudovirus screening assays were performed concurrently with the use of single reagent lots.

The cytotoxicity screen was performed using the Resazurin Cell Viability Assay Kit (AlamarBlue™) from Biotium. at different metadichol concentrations. In the cytotoxicity screen, 15 µL of HEK293T cells (3333 cells/µL) was added to each well along with 45 µL of media and 15 µL of metadichol dilution. After incubating for 16 hours, 7.5 µL of resazurin solution was added per the manufacturer's recommendations. The mixture was incubated for 1 hour before fluorescence was read using a 520 nm excitation filter and a 580–640 emission filter.

## Cytotoxicity Results

The cytotoxicity results image is available in the Figures carousel.

## Neutralization Results

**Absolute IC<sub>50</sub>**

**[Xaxis is the log (concentration)]**

The neutralization results image is available in the Figures carousel.

## Discussion

Research on zoonotic viruses has focused on GSPT1 (**G1 To S Phase Transition 1**), a host factor that is essential for viral replication (21). It is inhibited by the small molecule CC-90009 (22), which can reduce viral infection in human cells and reduce viral load and mortality in mice. G1 to S phase transition 1 (GSPT1) is a cellular protein that is involved in the termination of mRNA translation. GSPT1 has been shown to play a role in viral replication by interacting with viral proteins or cellular factors that affect viral infection. Some examples of viruses (23, -26) that use GSPT1 for replication include the following:

- **Ebola virus:** The Ebola virus is a zoonotic virus that can cause hemorrhagic fever in humans. Ebola virus polymerase, a viral protein that directs viral genome replication, hijacks GSPT1 to enhance its transcriptional activity. Targeting GSPT1 with a small-molecule degrader can inhibit Ebola virus infection in human cells
- **Hepatitis B virus:** The Hepatitis B virus is a DNA virus that can cause liver disease in humans. The hepatitis B virus core protein, a viral protein that forms the capsid of the virus, binds to GSPT1(24) and promotes its degradation. This reduces the stability of viral mRNA and inhibits viral replication.
- **Human immunodeficiency virus:** The human immunodeficiency virus (HIV) is a retrovirus that can cause acquired immunodeficiency syndrome (AIDS) in humans. The human immunodeficiency virus Tat protein, a viral protein that enhances viral transcription, interacts with GSPT1 and increases its transcriptional activity. This facilitates viral replication.

Understanding the molecular mechanisms and interactions of GSPT1 and its transcriptional regulators may provide novel insights and therapeutic targets for various diseases. (27–28)

PPAR $\gamma$  (**Peroxisome proliferator-activated receptor gamma**), a nuclear receptor that regulates lipid metabolism and inflammation, can bind to and inhibit GSPT1(29), leading to the suppression of protein synthesis and the induction of cell death in cancer cells. Understanding the molecular mechanisms and interactions of GSPT1 and its transcriptional regulators may provide novel insights and therapeutic targets for various diseases.

SP1 is a transcription factor that regulates the expression of genes involved in various cellular processes, such as cell growth, differentiation, and apoptosis. SP1 can bind to the promoter region of CRBN and activate its transcription. SP1 can bind to the promoter region of GSPT1 and activate its transcription (30, 31)

By controlling SP1 expression, one can control GSPT1 expression.

- **c-MYC (MYC Proto-Oncogene, BHLH Transcription Factor)** is a transcription factor that regulates genes involved in cell growth, proliferation, and metabolism. MYC binds to the SP1 promoter (32) and represses its expression, thereby modulating the activity of SP1 target genes. Thus, if we can control the expression of MYC, then SP1 expression is repressed, and GSPT1 expression is blocked. Metadichol binds to the vitamin D receptor (33), which controls the expression of c-MYC. (34) In

addition, metadichol induces the expression of PPAR gamma in stem and somatic cells (35) and can also repress GSPT1.

We propose that the mechanism of action of the inactivation of these 3 zoonotic viruses occurs through binding to VDR( Vitamin D receptor), which controls the expression of c-MYC by repressing SP1 and thus GSPT1. It is also likely that PPARG can be expressed to control the expression of GSPT1. Both mechanisms may operational in inhibiting viral replication.

Metadichol already inhibits (see ref 7) a multitude of viruses, as shown in Fig. 1. Given that metadichol is commercially available and nontoxic (35–37), this opens the way for direct testing in humans and hopeful mitigation of the threat to human life posed by zoonotic viruses.

## Declarations

**Conflict of interest:** The author declares that he is the founder and CEO of Nanorx Inc. and a major shareholder.

**Author Contributions:** All the work was planned and supervised by the author (PPR), who is solely responsible for its content. Funding was from the R & D budget of Nanorx Inc.

**Availability of data:** All the data are included in the manuscript and in the supplementary material provided

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## Figures

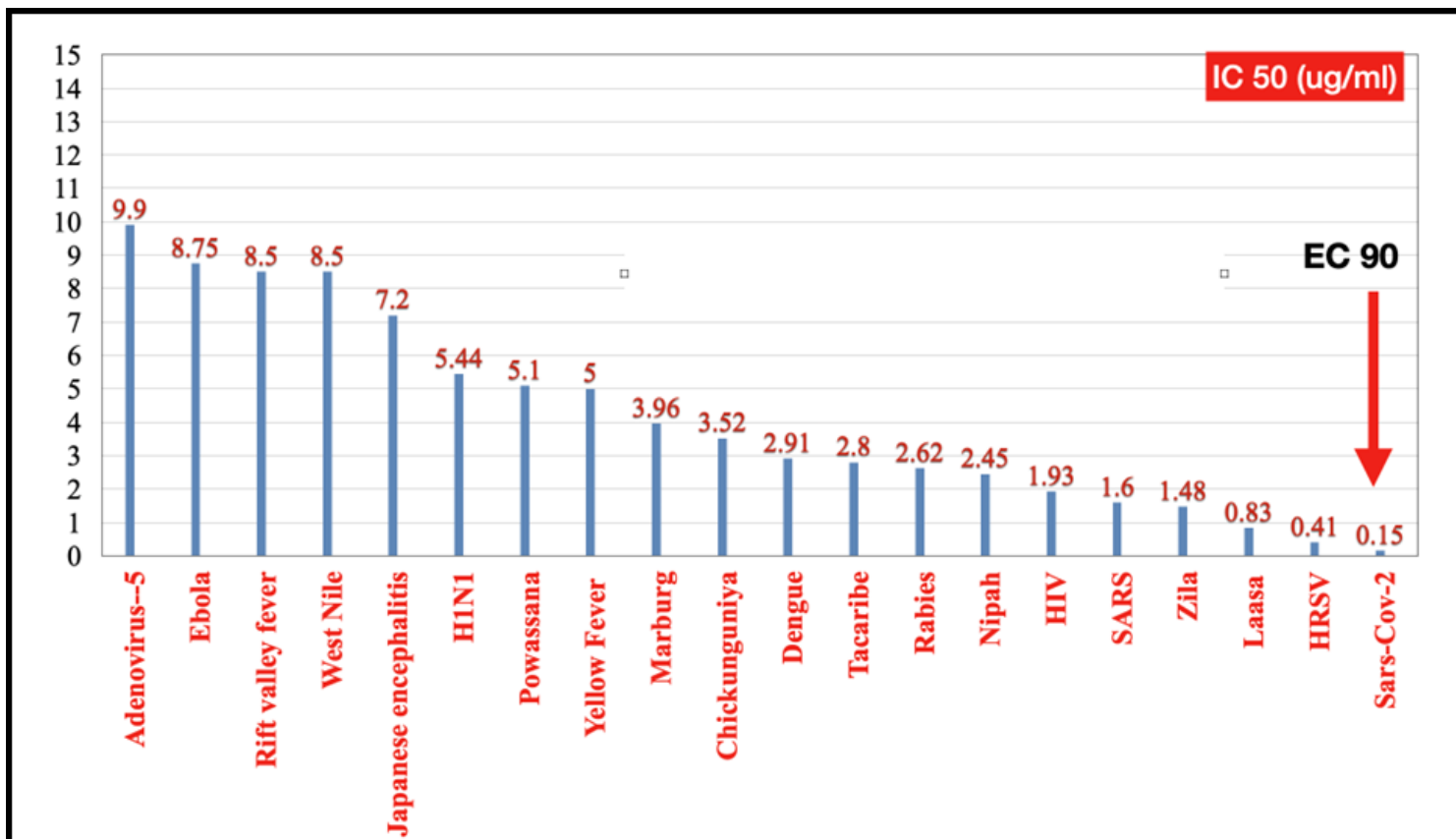
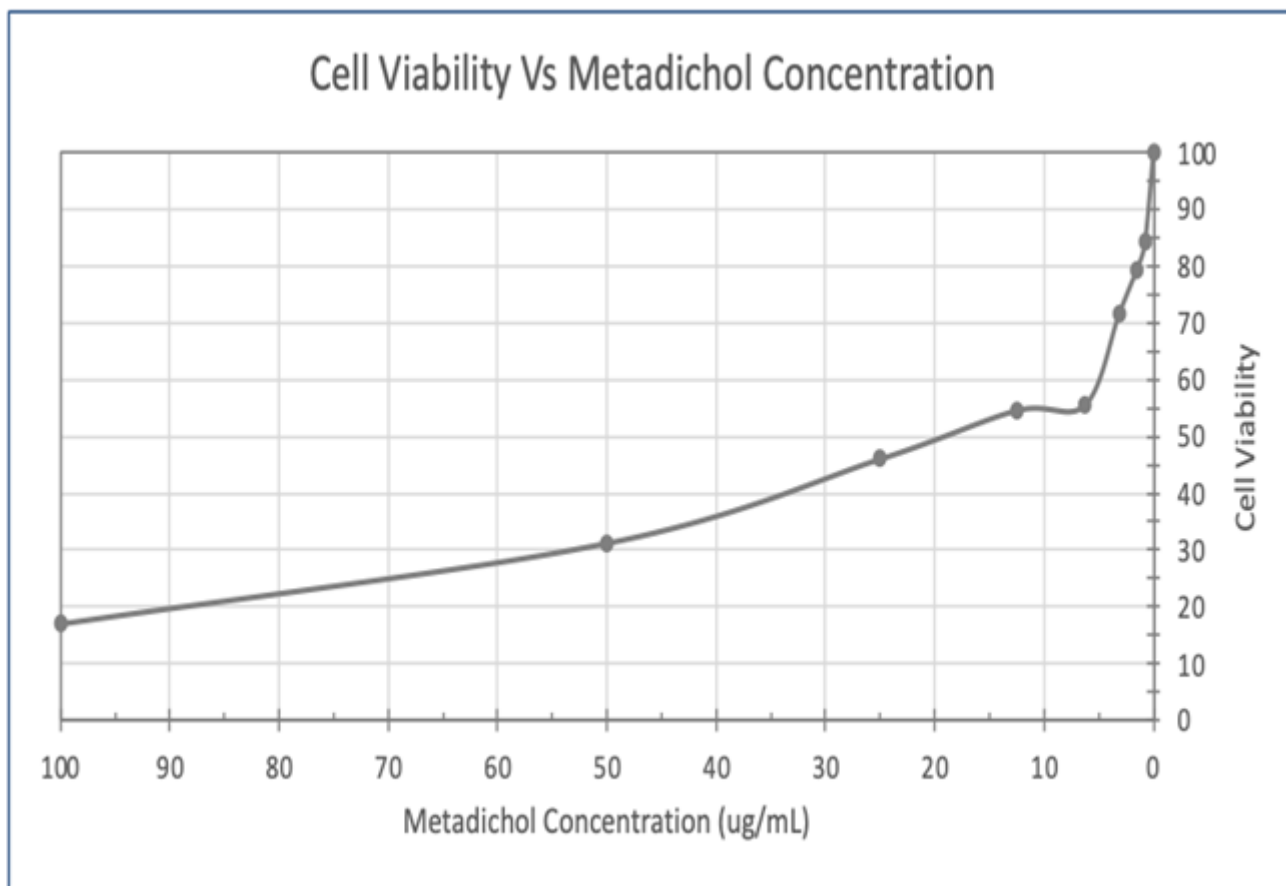


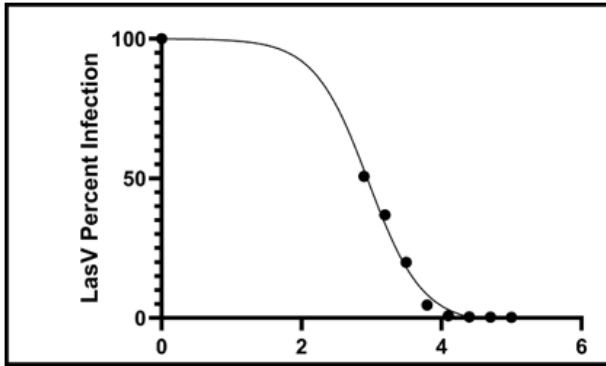
Figure 1

Metadichol and virus inhibition



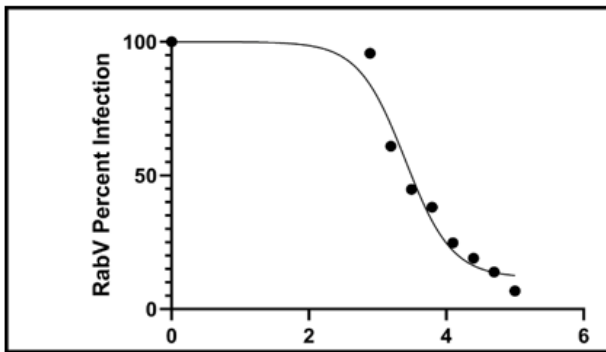
**Figure 2**

Unnumbered image in the Cytotoxicity Results section.



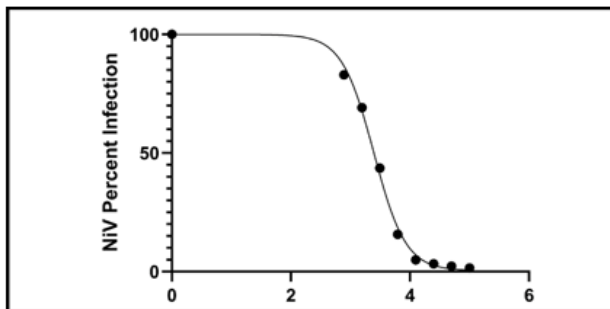
Laasa

Metadichol ng/ml



Rabies

Metadichol ng/ml



Nipah

Metadichol ng/ml

Figure 3

Unnumbered image in the Neutralization Results section.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [DataAntibodyScreening.docx](#)
- [MetadicholRawData.xlsx](#)
- [MetadicholInvitoCalculations.xlsx](#)