

## Antiviral Small Molecule Extracted from Plants

### Overview

<b>Drug Name</b>	Berbamine analogues
<b>Description</b>	<p>Positive-sense single-stranded RNA ((+)ssRNA) viruses are among the leading causes of human and animal infectious diseases in the world. Berbamine is a natural compound isolated from herbs and is safe to be used in humans. In a number of studies, berbamine was showed to have a broad-spectrum anti-(+)ss RNA viral activity.</p> <p>Considering that the current Covid-19 pandemic has created a global health and economic crisis, berbamine analogues are being developed into an effective therapeutic agent for the prevention or treatment of coronaviruses.</p>
<b>Target</b>	Transient receptor potential mucolipin family
<b>Drug Modality</b>	Small molecule
<b>Indication</b>	Coronaviruses, flaviviruses, enteroviruses
<b>Product Category</b>	Botanical
<b>Mechanism of Action</b>	Compromising the endolysosomal trafficking of viral receptors
<b>Status</b>	IND
<b>Patent</b>	Granted

### Collaboration Opportunity

Protheragen Inc. is actively seeking partnership for berbamine analogues. Potential collaboration can be strategic alliance, licensing, or marketing agreement.

We look forward to hearing from you.

### Target

#### Transient Receptor Potential Cation Channel, Mucolipin Subfamily (TRPML)

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The transient receptor potential (TRP) superfamily is comprised of 28 mammalian cation channels and is classified into six subfamilies, including TRPA (ankyrin), TRPV (vanilloid), TRPM (melastatin), TRPC (canonical), TRPP (polycystin), and TRPML (mucolipin).

TRPMLs are ion channels expressed in intracellular endosomes and lysosomes. The three proteins TRPML1, TRPML2 and TRPML3 are encoded by the mucolipin-1 (MCOLN1), mucolipin-2 (MCOLN2) and mucolipin-3 (MCOLN3) genes, respectively. TRPML1, TRPML2, and TRPML3 are localized in the lysosome-associated membrane protein (Lamp-1)+ or Rab7+ late endosomal and lysosomal (LEL) compartment. These channels are tetramers, consisting of proteins with six transmembrane-spanning domains and amino- and carboxy-terminal tails oriented toward the cytosol. Three TRPML subtypes share about 40% of amino acid sequence identity.

All TRPMLs could be activated by phosphatidylinositol-3,5-biphosphate (PtdIns(3, 5)P<sub>2</sub>) and play a role in membrane trafficking autophagy, exocytosis, and ion homeostasis.

## Drug Modality

### Small Molecule

Libraries of natural compounds are screened through high-content fluorescence imaging platform to identify more efficient and specific endosomal trafficking modulators. Several bis-benzylisoquinoline alkaloids, especially berbamine, were found to potently suppress the infection of many positive-sense single-stranded RNA viruses. Berbamine analogues are being developed into next generation antiviral small molecule.

## Indication

### Coronaviruses

Positive-sense single-stranded RNA viruses, including coronaviruses, flaviviruses, and enteroviruses, are a leading cause of human and animal infectious diseases around the world. As a new coronavirus, SARS-CoV-2 causes febrile respiratory illness and was officially named coronavirus disease 2019 (Covid-19) by WHO.

Following the initial leap across the species barrier, SARS-CoV-2 spreads rapidly among humans and plagued the whole world within three months. According to WHO, as of September 7, 2020, more than 27 million laboratory-confirmed cases and 881,464 deaths from Covid-19 worldwide have been reported. There is currently no effective clinical treatment against Covid-19.

## Mechanism of Action

### Compromising Endolysosomal Trafficking to Inhibit the Virus Infection

The activation of TRPMLs via PI(3,5)P2 can trigger the release  $Ca^{2+}$  from endosomes and lysosomes, which participates in various endolysosomal trafficking events, including trafficking of endosomal vesicles, fusion of late endosomes and lysosomes, and lysosome-mediated exocytosis.

Berbamine could inhibit the activity of TRPMLs to compromise the endolysosomal trafficking of membrane receptors for (+)ss RNA viruses. The accumulation of endosomes leads to an increased level of secretion of these receptors via extracellular vesicles and a concomitant decrease in the virus receptor levels at the plasma membrane. The decrease in the amount of virus receptors renders the cell resistant to virus infection. Thus, berbamine prevents the entry of (+)ss RNA viruses in host cells.

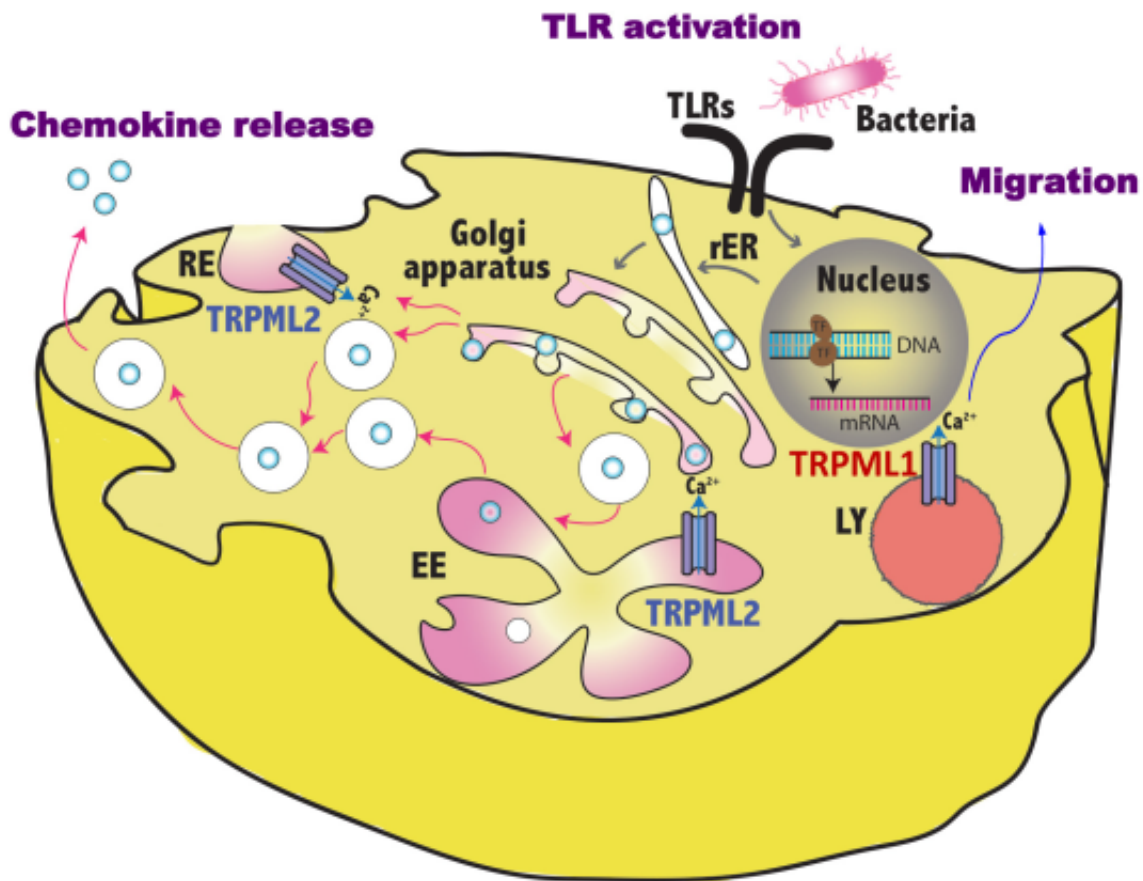


Figure. Schematic representation of TRPMLs involvement in the regulation of vesicles trafficking induced by pathogen sensing.

Front Immunol. 2020 Apr 29;11:739.

## Status

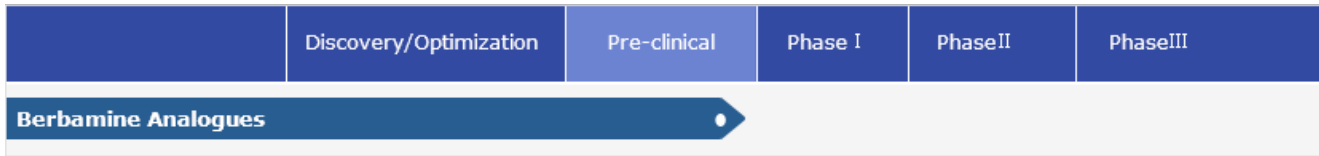
### The Status of Berbamine Analogues

Preclinical studies have been completed. The IND approvals have been received for the berbamine analogues. Global or regional rights are available for out-licensing.

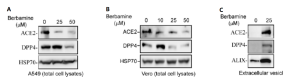
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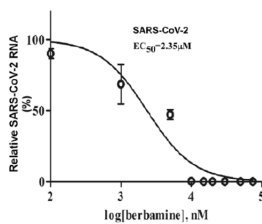


## Data



### The Effect of Berberamine on the Levels of Viral Receptors

A549 cells or Vero cells in the presence of cycloheximide were treated with/without berberamine for 6 h, after which the cell lysates were subjected to ACE2 or DPP4 immunoblotting. Extracellular vesicles were collected from the culture medium of control or berberamine treated A549 cells, and the levels of ACE2, DPP4, and ALIX in these extracellular vesicles were determined by immunoblotting. These results suggested that berberamine inhibits the endolysosomal trafficking of the plasma membrane receptors of (+)ss RNA viruses.



### The Anti- SARS-CoV-2 Activity of Berberamine

Vero E6 cells were treated with berberamine at the indicated concentrations for 3 h, and were then infected with SARS-CoV-2. Cell lysates were collected and subjected to RT-PCR quantification of SARS-CoV-2 RNA. The result showed that berberamine significantly inhibited the viral yield.