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Lactimidomycin is a broad-spectrum inhibitor of dengue and other RNA viruses

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Abstract

Dengue virus, a member of the *Flaviviridae* family, is a mosquito-borne pathogen and the causative agent of dengue fever. Despite the nearly 400 million new infections estimated annually, no vaccines or specific antiviral therapeutics are currently available. We identified lactimidomycin (LTM), a recently established inhibitor of translation elongation, as a potent inhibitor of dengue virus 2 infection in cell culture. The antiviral activity is observed at concentrations that do not affect cell viability. We show that Kunjin virus and Modoc virus, two other members of the *Flaviviridae* family, as well as vesicular stomatitis virus and poliovirus 1, are also sensitive to LTM. Our findings suggest that inhibition of translation elongation, an obligate step in the viral replication cycle, may provide a general antiviral strategy against fast-replicating RNA viruses.

Keywords

Lactimidomycin; translation inhibitor; antiviral; dengue virus; broad spectrum; host-targeted antiviral

Dengue virus 2 (DENV2), a member of the *Flaviviridae* family, is an enveloped, positive-strand RNA virus and the causative agent of dengue fever. Dengue infection can be serious, potentially leading to hemorrhagic fever, shock syndrome, and death. It is estimated that over 350 million people are infected annually and a third of the world's population is at risk (Bhatt et al., 2013). Despite these staggering numbers, there is currently no vaccine, nor antiviral drugs available to prevent or to treat infection. The development of vaccines has been challenging due to the diversity of DENV serotypes and the occurrence of antibody-dependent enhancement of infection, a phenomenon in which neutralizing antibodies against one DENV serotype can exacerbate disease upon subsequent infection with another serotype (Guzman et al., 2013; Murphy and Whitehead, 2011).

Research and development of antivirals to fight DENV are therefore of great interest. Due to the intrinsically high mutation rate of RNA viruses, resistance to antiviral drugs that act

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against viral targets (e.g., inhibitors of viral proteases and polymerases) can occur rapidly. To complement traditional antivirals, agents that act via host targets and that present higher barriers to resistance have become of increasing interest (for review see (Noble et al., 2010)). Since the immune system clears DENV and other acute viral pathogens if given sufficient time, the goal of antiviral therapy against these pathogens may be to shorten the duration of the infection and decrease viral burden by inhibiting replication, thereby reducing transmission and the incidence of severe disease. Celgosivir and other inhibitors of host alpha-glucosidases are examples illustrating the potential of this strategy. These compounds potently inhibit DENV replication, reduce disease, and improve survival in murine models (Perry et al., 2013; Rathore et al., 2011; Watanabe et al., 2016; Watanabe et al., 2012; Whitby et al., 2005); moreover, the genetic barrier to resistance against one such inhibitor, UV-4B, appears to be high (Plummer et al., 2015). Celogosivir's safety and efficacy were demonstrated in a phase Ib trial (Low et al., 2014), and a new phase Ib/IIa trial (NCT02569827) has been approved to investigate an altered dosing regimen. Heralded by this work, additional strategies for inhibiting DENV and other RNA viruses via host targets through repurposing of known drugs or validation of new targets and antiviral entities are of considerable interest.

All viruses lack their own translational apparatus and rely entirely upon the host cell's protein synthesis machinery. Indeed, André Lwoff noted the absence of ribosomes and the cellular translation machinery as a defining feature of viruses (Lwoff, 1957). The translation process consists of three steps: initiation, elongation, and termination. The initiation step is highly regulated and leads to formation of an elongation-competent 80S ribosomal complex. For most cellular mRNAs, this process is dependent upon the presence of a 5' cap on the mRNA. Binding of eIF4F to the 5' cap enables recruitment of the 40S ribosomal subunit to the mRNA. This is followed by the highly regulated, sequential loss of eIF2-bound GDP, recruitment eIF5B-GTP and the 60S ribosomal subunit, and loss of eIF5B-GDP and eIF1A to yield an elongation-competent 80S ribosomal complex (for review (Jackson et al., 2010)).

While some viruses accomplish translation initiation via cap-dependent mechanisms, they do so via a myriad of mechanisms with varying utilization of host eukaryotic initiation factors (eIFs). Other viruses initiate at internal ribosome entry sites (IRES) via cap-independent mechanisms (for review see (Walsh et al., 2013)). The elongation step results in polymerization of amino acids to synthesize polypeptides as templated by the mRNA template. Elongation requires (1) delivery of the correct aminoacyl-tRNA to the A-site by eEF1A; (2) formation of the new peptide bond, which transfers the nascent peptide to the A-site tRNA; and (3) eEF2-catalyzed transfer of this new peptidyl-tRNA to the P-site and transfer of the deacetylated tRNA to the E site, thereby freeing the A-site for the next aminoacyl-tRNA (Richter and Collier, 2015; Schneider-Poetsch et al., 2010b). All known viruses rely on cellular elongation factors for expression of the viral genome. For the *Flaviviridae* and other positive-strand RNA viruses, translation of the viral genome is an especially critical control point in the replication cycle. For DENV, translation efficiency has been shown to be a determinant of productive infection (Edgil et al., 2003).

Lactimidomycin (LTM) (Fig. 1A) is a natural product isolated from *Streptomyces amphibiosporus* (ATCC53964) that inhibits translation elongation through binding to the

ribosome E-site. This prevents the ribosome from leaving the start site and blocks the very first round of elongation (Ju et al., 2005; Schneider-Poetsch et al., 2010a; Schneider-Poetsch et al., 2010b). Cycloheximide (CHX) also blocks translation elongation by binding in the E-site, but its smaller size permits binding of one tRNA in the E-site before elongation is halted. The absolute dependence of viruses upon translation elongation has stimulated interest in this process as a source of antiviral targets. Studies performed in the 1980s examined the use of cycloheximide as an inhibitor for encephalomyocarditis virus and vesicular stomatitis virus (VSV) (Ramabhadran and Thach, 1980; Yau et al., 1978); however, CHX's effects on transcription along with other toxic effects have been prohibitive for its development as a drug. LTM inhibits translation elongation with ten-fold greater potency than CHX while lacking CHX's effects on transcription even at high concentrations (Schneider-Poetsch et al., 2010a). This has stimulated considerable interest in evaluating LTM and related glutarimides as anticancer agents (Larsen et al., 2015; Micoine et al., 2013) and prompted us to examine LTM's potential as an anti-DENV agent.

Huh7 cells infected with DENV serotype 2 New Guinea C (DENV2 NGC) at a multiplicity of infection (MOI) of 1 were treated with varying concentrations of LTM for twenty-four hours, corresponding to a single round of infection (Fig. 1B). The cytotoxicity of LTM was evaluated in parallel to control for potential indirect antiviral effects due to a decrease in cell viability. LTM induced a clear dose-responsive inhibition of DENV2 infectious particle production (Fig 1B black circles) with an EC₉₀ value – defined as the concentration of inhibitor needed to reduce the single-cycle viral yield by 10-fold -- of 0.4 μ M, as determined by non-linear fit of the data. No measurable decrease in cell viability was detected at concentrations up to 12.5 μ M (Fig 1B red triangles). While statistically significant cytotoxicity ($p < 0.05$) was observed in both DENV2-infected and non-infected cells at LTM concentrations above 25 μ M (Fig. 1C), we were unable to determine the CC₅₀ of LTM, as a lower plateau was not reached even at 200 μ M.

To assess the effects of LTM on translation and replication of the DENV2 genomic RNA, we utilized a replicon system in which the viral structural proteins are replaced by a luciferase reporter, thus permitting analysis of viral RNA translation and replication in the absence of viral entry, assembly, and egress (Clyde et al., 2008). In this system, luciferase activity served as a readout for translation of the input replicon RNA at early times following electroporation (< 24 hours) and as a marker of both viral translation and replication of the viral RNA at later time points ((Carocci et al., 2015) and Supplemental Methods). LTM inhibited DENV2-Fluc^{WT} translation at 0.5 μ M, as evidenced by a profound decrease in luciferase activity at 6 hours post-electroporation (Fig. 2A). In experiments utilizing infectious DENV2 NGC, delaying LTM treatment until 12 hours post-infection (12 hpi) allowed some translation of DENV2 protein to occur as observed by immunofluorescence staining for the DENV E protein in cells (Fig. 2D) Consistent with this, steady-state replication of the DENV2 RNA as detected by qRT-PCR analysis (Fig. 2B) and infectious virus particle production (Fig. 2C) were also partially restored when LTM treatment was delayed to 12 hpi. Together, these data show that LTM is a potent inhibitor of DENV2 and suggest that LTM's inhibition of DENV2 translation leads to reduced production of newly infectious particles.

In order to determine whether the antiviral activity of LTM extends to other viruses, we first infected Vero cells with Kunjin virus and Modoc virus, two other members of the *Flaviviridae* family, at an MOI of 1 in the presence or absence of the indicated concentration of LTM. Twenty-four hours later, supernatants were harvested to allow quantification of viral yield (Fig. 3A-B). Both viruses were potently inhibited by LTM. Notably, visual inspection of the cells indicated that this decrease in viral replication is not due to cytopathic effects and cell death (data not shown), consistent with the cell viability studies.

We then tested RNA viruses outside of the *Flaviviridae* family: poliovirus 1 (PV1) from the *Picornaviridae* family and vesicular stomatitis virus (VSV) from the *Rhabdoviridae* family. PV1 is a non-enveloped, positive single-strand RNA virus whose genome undergoes IRES-dependent translation (Sweeney et al., 2014). VSV is an enveloped, negative single-strand RNA virus that utilizes cap-dependent translation of its mRNAs (Lee et al., 2013). Both PV1 and VSV are fast-replicating viruses. Vero cells were infected with PV1 or VSV at an MOI of 1 and treated with the indicated concentration of LTM for 6 hours, after which viral yields were determined by plaque formation assays. We found that, as for the *Flaviviridae*, LTM greatly inhibits production of infectious particles of PV1 and VSV (Fig. 3C-D, respectively). Interestingly, within 6 hpi in cell culture, PV1 and VSV infections result in major cytopathic effects. We observed condensed nuclei (arrow in enlarged picture Supplementary Fig. 1) characteristic of the canonical cytopathic effects previously described for PV1 (Agol et al., 2000), and apoptotic cells and apoptotic bodies in VSV-infected cells treated with DMSO (arrow head in enlarged picture Supplemental Fig. 1) (Gadaleta et al., 2005). In contrast, cells treated with LTM showed no cytopathic effects (Supplemental Fig. 1). This suggests that LTM may protect cells from viral cytopathic effects including apoptosis, likely through inhibition of virus protein production and replication. Since inhibition of translation by CHX has been shown to protect cells from apoptosis following ischemia-induced injury (Lepran et al., 1982; Liang et al., 2003; Musat-Marcu et al., 1999), it is possible that LTM's cytoprotective effect is due to both inhibition of VSV and PV1 replication as well as inhibition of apoptosis.

Collectively, our data show that LTM is a potent and non-toxic inhibitor of DENV and other RNA viruses *in cellulo*. Although further characterization is necessary to test whether LTM could be used as a broad spectrum antiviral *in vivo*, its potency and defined mechanism of action should make it a useful tool in reevaluation of the inhibition of viral translation as a potential antiviral strategy. Such a strategy is attractive for several reasons. First, translation is an obligate, host-dependent step in the replication cycle of all viruses. Second, although viruses are completely dependent upon the host translational machinery, their use of diverse mechanisms for translation initiation suggests that there may be untapped mechanisms for selectively inhibiting virus versus host translation. Indeed, Nature itself utilizes this strategy, as evidenced by the phosphorylation of eIF2A by protein kinase RNA-activated (PKR) upon its activation by double-stranded RNA, which leads to inhibition of translation initiation at canonical AUG sites. Third, although cycloheximide's reversibility and poor tolerability have precluded its development as a drug (Gosselin R.E., 1984; Lewis, 1996), lactimidomycin's higher potency and selectivity may provide a more favorable safety profile, as suggested by two studies assessing LTM's efficacy as an anti-tumor agent in murine xenograft models. In the first study, LTM given at 0.25 mg/kg for nine days significantly extended survival in a

murine model of P388 leukemia (Sugawara et al., 1992). While *in vivo* toxicity was not explicitly measured, the lethal dose was reported as 32 mg/kg (Q1D, 3 i.p.) in the tumor-bearing mice. More recently, LTM given at 0.6 mg/kg daily for one month inhibited growth of an MDA MB 231 cell xenograft without reported toxicity (Schneider-Poetsch et al., 2010a).

We also note that while cellular translation is necessary for the host antiviral response, we believe that potent but transient inhibition of protein production (viral and host) may impair and/or delay the spread of fast-replicating viral pathogens. This may be beneficial for the host. Fast-replicating viruses usually induce a strong innate immune response; however, pathogenesis including tissue damage ensue because viral spread occurs much more rapidly than induction of the immune response. Since disease severity is correlated with high viral load and massive cytokine production, an ideal antiviral agent may be one that can inhibit viral replication and spread while also modulating the host response to limit immunopathogenic mechanisms underlying severe dengue without blunting the host responses needed for viral clearance. Potentially consistent with this, we observed that LTM protected human fibroblast cells from Sendai virus-associated cytopathic effects without changes in the induction of the interferon response, as reflected by quantification of interferon β (IFN β) and interferon-stimulated gene 54 (ISG54) transcripts (unpublished results). Agents such as LTM, with the potential to limit cytokine production and viral replication while still possibly permitting the induction of immune responses responsible for viral clearance, are therefore worth consideration.

Materials and Methods

Detailed materials and methods are provided in Supplementary information online.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DENV2	dengue virus 2
VSV	vesicular stomatitis virus
PV1	poliovirus 1
LTM	Lactimidomycin
CHX	Cycloheximide

MPA	mycophenolic acid
PFU	plaque-forming unit
FFU	focus-forming unit
EMCV	encephalomyocarditis virus

References

- Agol VI, Belov GA, Bienz K, Egger D, Kolesnikova MS, Romanova LI, Sladkova LV, Tolskaya EA. Competing death programs in poliovirus-infected cells: commitment switch in the middle of the infectious cycle. *J Virol.* 2000; 74:5534–5541. [PubMed: 10823859]
- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI. The global distribution and burden of dengue. *Nature.* 2013; 496:504–507. [PubMed: 23563266]
- Carocci M, Hinshaw SM, Rodgers MA, Villareal VA, Burri DJ, Pilankatta R, Maharaj NP, Gack MU, Stavale EJ, Warfield KL, Yang PL. The bioactive lipid 4-hydroxyphenyl retinamide inhibits flavivirus replication. *Antimicrob Agents Chemother.* 2015; 59:85–95. [PubMed: 25313218]
- Clyde K, Barrera J, Harris E. The capsid-coding region hairpin element (cHP) is a critical determinant of dengue virus and West Nile virus RNA synthesis. *Virology.* 2008; 379:314–323. [PubMed: 18676000]
- Edgil D, Diamond MS, Holden KL, Paranjape SM, Harris E. Translation efficiency determines differences in cellular infection among dengue virus type 2 strains. *Virology.* 2003; 317:275–290. [PubMed: 14698666]
- Gadaleta P, Perfetti X, Mersich S, Coulombie F. Early activation of the mitochondrial apoptotic pathway in Vesicular Stomatitis virus-infected cells. *Virus Res.* 2005; 109:65–69. [PubMed: 15826914]
- Gosselin RE, S.R.P. Hodge HC. *Clinical Toxicology of Commercial Products* (5th ed.). 1984
- Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. *Arch Virol.* 2013; 158:1445–1459. [PubMed: 23471635]
- Jackson RJ, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol.* 2010; 11:113–127. [PubMed: 20094052]
- Ju J, Lim SK, Jiang H, Shen B. Migrastatin and dorrigocins are shunt metabolites of iso migrastatin. *J Am Chem Soc.* 2005; 127:1622–1623. [PubMed: 15700980]
- Larsen BJ, Sun Z, Lachacz E, Khomutnyk Y, Soellner MB, Nagorny P. Synthesis and Biological Evaluation of Lactimidomycin and Its Analogues. *Chemistry.* 2015; 21:19159–19167. [PubMed: 26577990]
- Lee AS, Burdeinick-Kerr R, Whelan SP. A ribosome-specialized translation initiation pathway is required for cap-dependent translation of vesicular stomatitis virus mRNAs. *Proc Natl Acad Sci U S A.* 2013; 110:324–329. [PubMed: 23169626]
- Lepran I, Koltai M, Szekeres L. Effect of actinomycin D and cycloheximide on experimental myocardial infarction in rats. *Eur J Pharmacol.* 1982; 77:197–199. [PubMed: 7060637]
- Lewis RJ. *Sax's Dangerous Properties of Industrial Materials* (9th ed.). 1996
- Liang CL, Yang LC, Lu K, Hsu HC, Cho CL, Chen SD, Huang HY, Chen HJ. Neuroprotective synergy of N-methyl-D-aspartate receptor antagonist (MK801) and protein synthesis inhibitor (cycloheximide) on spinal cord ischemia-reperfusion injury in rats. *J Neurotrauma.* 2003; 20:195–206. [PubMed: 12675972]
- Low JG, Sung C, Wijaya L, Wei Y, Rathore AP, Watanabe S, Tan BH, Toh L, Chua LT, Hou Y, Chow A, Howe S, Chan WK, Tan KH, Chung JS, Chheng BP, Lye DC, Tambayah PA, Ng LC, Connolly J, Hibberd ML, Leo YS, Cheung YB, Ooi EE, Vasudevan SG. Efficacy and safety of celgosivir in

- patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. *Lancet Infect Dis.* 2014; 14:706–715. [PubMed: 24877997]
- Lwoff A. The concept of virus. *J Gen Microbiol.* 1957; 17:239–253. [PubMed: 13481308]
- Micoine K, Persich P, Llaveria J, Lam MH, Maderna A, Loganzo F, Furstner A. Total syntheses and biological reassessment of lactimidomycin, isomigrastatin and congener glutarimide antibiotics. *Chemistry.* 2013; 19:7370–7383. [PubMed: 23595541]
- Murphy BR, Whitehead SS. Immune response to dengue virus and prospects for a vaccine. *Annu Rev Immunol.* 2011; 29:587–619. [PubMed: 21219187]
- Musat-Marcu S, Gunter HE, Jugdutt BI, Docherty JC. Inhibition of apoptosis after ischemia reperfusion in rat myocardium by cycloheximide. *J Mol Cell Cardiol.* 1999; 31:1073–1082. [PubMed: 10336845]
- Noble CG, Chen YL, Dong H, Gu F, Lim SP, Schul W, Wang QY, Shi PY. Strategies for development of Dengue virus inhibitors. *Antiviral Res.* 2010; 85:450–462. [PubMed: 20060421]
- Perry ST, Buck MD, Plummer EM, Penmasta RA, Batra H, Stavale EJ, Warfield KL, Dwek RA, Butters TD, Alonzi DS, Lada SM, King K, Klose B, Ramstedt U, Shresta S. An iminosugar with potent inhibition of dengue virus infection in vivo. *Antiviral Res.* 2013; 98:35–43. [PubMed: 23376501]
- Plummer E, Buck MD, Sanchez M, Greenbaum JA, Turner J, Grewal R, Klose B, Sampath A, Warfield KL, Peters B, Ramstedt U, Shresta S. Dengue Virus Evolution under a Host-Targeted Antiviral. *J Virol.* 2015; 89:5592–5601. [PubMed: 25762732]
- Ramabhadran TV, Thach RE. Specificity of protein synthesis inhibitors in the inhibition of encephalomyocarditis virus replication. *J Virol.* 1980; 34:293–296. [PubMed: 6246261]
- Rathore AP, Paradkar PN, Watanabe S, Tan KH, Sung C, Connolly JE, Low J, Ooi EE, Vasudevan SG. Celgosivir treatment misfolds dengue virus NS1 protein, induces cellular pro-survival genes and protects against lethal challenge mouse model. *Antiviral Res.* 2011; 92:453–460. [PubMed: 22020302]
- Richter JD, Collier J. Pausing on Polyribosomes: Make Way for Elongation in Translational Control. *Cell.* 2015; 163:292–300. [PubMed: 26451481]
- Schneider-Poetsch T, Ju J, Eyler DE, Dang Y, Bhat S, Merrick WC, Green R, Shen B, Liu JO. Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. *Nat Chem Biol.* 2010a; 6:209–217. [PubMed: 20118940]
- Schneider-Poetsch T, Usui T, Kaida D, Yoshida M. Garbled messages and corrupted translations. *Nat Chem Biol.* 2010b; 6:189–198. [PubMed: 20154667]
- Sugawara K, Nishiyama Y, Toda S, Komiyama N, Hatori M, Moriyama T, Sawada Y, Kamei H, Konishi M, Oki T. Lactimidomycin, a new glutarimide group antibiotic. Production, isolation, structure and biological activity. *J Antibiot (Tokyo).* 1992; 45:1433–1441. [PubMed: 1429229]
- Sweeney TR, Abaeva IS, Pestova TV, Hellen CU. The mechanism of translation initiation on Type 1 picornavirus IRESs. *EMBO J.* 2014; 33:76–92. [PubMed: 24357634]
- Walsh D, Mathews MB, Mohr I. Tinkering with translation: protein synthesis in virus-infected cells. *Cold Spring Harb Perspect Biol.* 2013; 5:a012351. [PubMed: 23209131]
- Watanabe S, Chan KW, Dow G, Ooi EE, Low JG, Vasudevan SG. Optimizing celgosivir therapy in mouse models of dengue virus infection of serotypes 1 and 2: The search for a window for potential therapeutic efficacy. *Antiviral Res.* 2016; 127:10–19. [PubMed: 26794905]
- Watanabe S, Rathore AP, Sung C, Lu F, Khoo YM, Connolly J, Low J, Ooi EE, Lee HS, Vasudevan SG. Dose- and schedule-dependent protective efficacy of celgosivir in a lethal mouse model for dengue virus infection informs dosing regimen for a proof of concept clinical trial. *Antiviral Res.* 2012; 96:32–35. [PubMed: 22867971]
- Whitby K, Pierson TC, Geiss B, Lane K, Engle M, Zhou Y, Doms RW, Diamond MS. Castanospermine, a potent inhibitor of dengue virus infection in vitro and in vivo. *J Virol.* 2005; 79:8698–8706. [PubMed: 15994763]
- Yau PM, Godefroy-Colburn T, Birge CH, Ramabhadran TV, Thach RE. Specificity of interferon action in protein synthesis. *J Virol.* 1978; 27:648–658. [PubMed: 212587]

Highlights

- * Lactimidomycin inhibits dengue virus translation and reduces viral replication without cytotoxicity
- * Lactimidomycin also inhibits other members of the *Flaviviridae*, *Picornaviridae*, and *Rhabdoviridae* families
- * Inhibition of poliovirus and vesicular stomatitis virus is associated with protection for virus-induced cytopathic effects

A.

B.

C.

Figure 1. Lactimidomycin inhibits DENV2 production in Huh7 cells at concentrations that are non-cytotoxic

(A) Structure of lactimidomycin (LTM). (B) LTM reduces DENV2 replication in Huh7 cells infected at an MOI of 1 (black dots) within 24 hours without affecting cell viability (red open triangles). (C) Concentration-dependent effect of LTM on viability of DENV2-infected (red dots) and non-infected (black dots) Huh7 cells treated for 24 hours post-infection. Viral titers were determined by focus-forming assay (see Supplemental methods), and the cell viability was assessed with Cell Titer-Glo® assay. Experiments were performed in duplicates, the error bar represent the mean $\pm$ SD.

Figure 2. LTM inhibit DENV2 RNA translation

(A) Huh7 cells electroporated with subgenomic replicon RNA (DENV2-Fluc^{WT}) were used to analyze the effects of LTM on translation and replication of DENV2 RNA.

Cycloheximide (CHX, 30 µg/mL) was used as a control inhibitor of translation, and 5 µM mycophenolic acid (MPA) was used as a control for the inhibition of RNA replication.

Luciferase activity was read at 6, 24, 48, and 72 h after electroporation. LTM (0.5 µM) reduces translation of the input replicon RNA, as evidenced by decreased luciferase signal at 6 and 24 hours post-electroporation. (B-D) Huh7 cells were infected with DENV2 at an

MOI of 1 and treated with DMSO or 0.5 μ M LTM at indicated time post-infection. At 24 hpi, B) supernatants were harvested for viral titration; (C) cells were lysed, RNA extracted and subjected to RT-qPCR analysis to determine the relative amount of DENV2 RNA; and (D) cells were fixed and immunostained to examine DENV2 E protein expression. The merged images are shown with DAPI signal pseudocolored blue and DENV2 E pseudocolored red. These experiments were performed in duplicates, the error bars represent the mean $\pm$ SD. Representative immunofluorescence images are shown.

Figure 3. LTM inhibits other viruses

Vero cells were infected at an MOI of 1 with (A) Kunjin, (B) Modoc, (C) PV1, or (D) VSV and then treated with DMSO or 0.125, 0.5, or 2 μ M LTM. Culture supernatants were harvested at either 24 hpi (Kunjin and Modoc) or 6 hpi (VSV and PV1), and viral yields quantified by viral plaque forming assay. (A) Kunjin virus. (B) Modoc virus. (C) PV1. (D) VSV. Experiments were performed in duplicates. Data plotted are the mean $\pm$ SD.