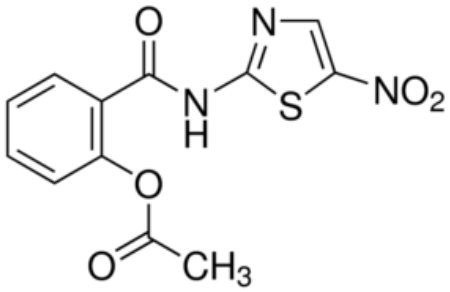
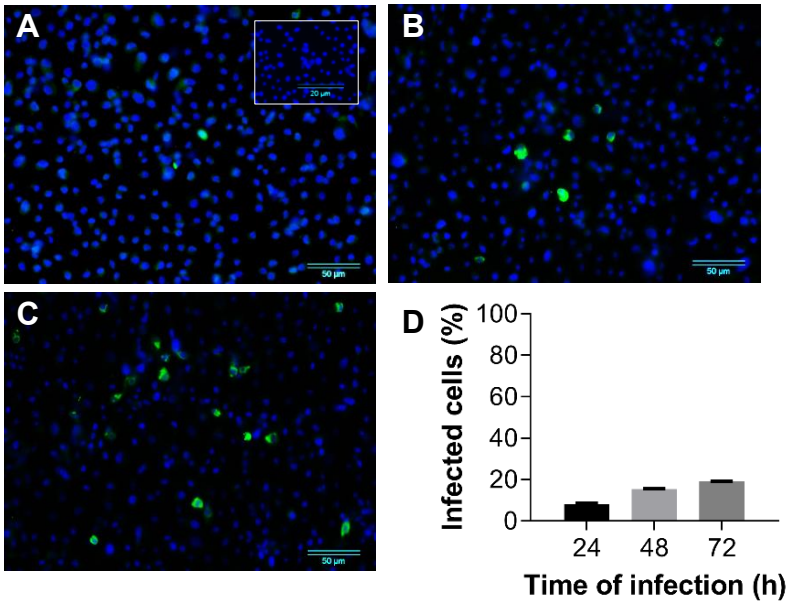
Supplemental material 1: Chemical structure of Nitazoxanide



Supplemental material 2: Nitazoxanide did not inhibit viral progeny production in C6/36 cells



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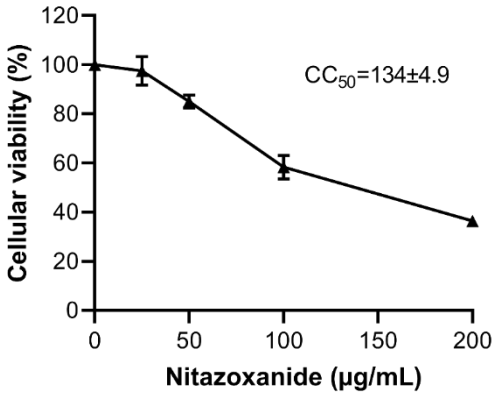

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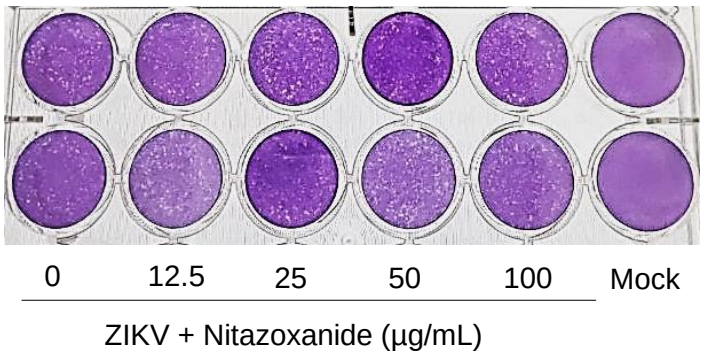
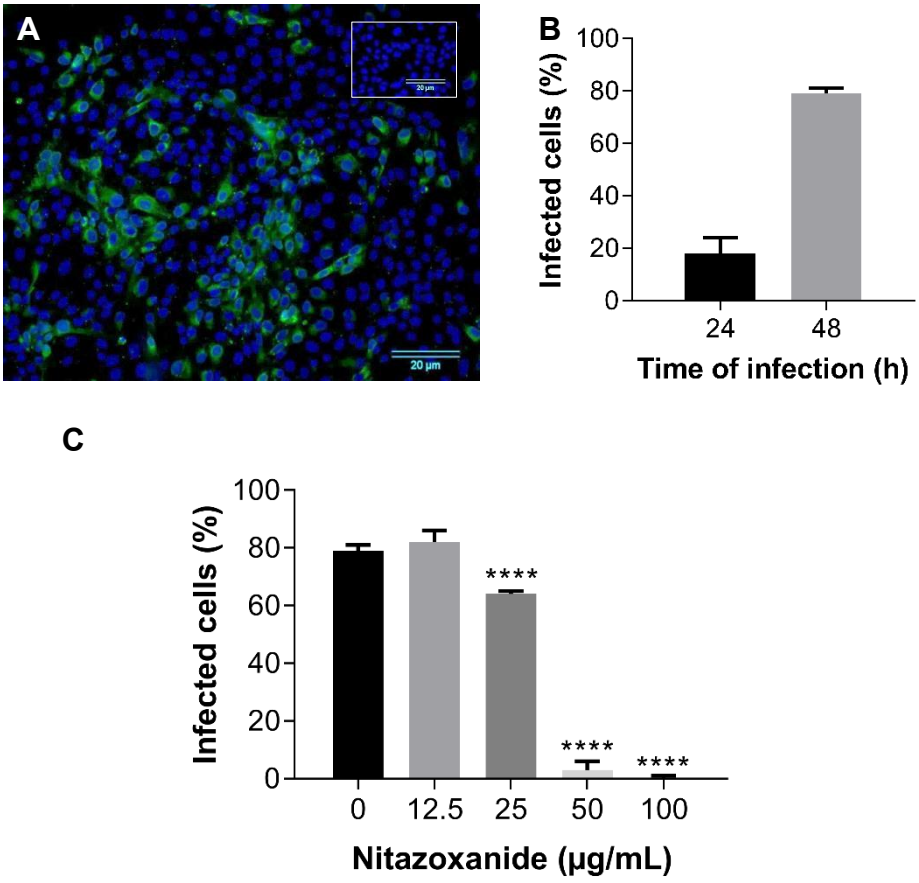


Supplemental material 3: Nitazoxanide displays antiviral activity against the dengue virus serotype 2



Supplemental Table 1. Primers, probe and curve used for ZIKV detection and quantification.

	Sequence
ZK-F-E-Bonn	AGYCGYTGYCCAACACAAG
ZK-R-E-Bonn	CACCARRCTCCCYTTGCCA
ZK -E probe	6-FAM6-CCTMCCTYGAYAAGCARTCAGACACYCAA-BHQ
ZK-E- Bonn curve	TTCGTCACCARRCTCCCYTTGCCACGTATTTGRGTGTCTGAYTGCTT
	RTCRAGGKAGGATGCGTCTTGTGTTGGRCARCGRCTCTGATA

Legends

Supplemental Figure 1. Chemical structure of Nitazoxanide (2-(Acetyloxy)-N-(5-nitro-2-thiazolyl) benzamide.

Supplemental figure 2. Infection kinetics, cellular viability and anti-ZIKV effect of nitazoxanide on C6/36 cells. The cultures were infected with ZIKV at a MOI of 10 for **(A)** 24h, **(B)** 48h and **(C)** 72h. The antiviral activity was evaluated 48h post-infection by immunofluorescence, indicating the viral antigen in green and the nucleus of the host cells in blue. **(D)** The graph represents the means ± standard deviations of the percentage of infected cells during infection kinetics. **(E)** Cellular viability was determined by the MTT assay 48h post-infection with NTZ up to 200 µg/mL. The graph represents the means ± standard deviations of the percentage of cellular viability. **(F)** The reduction of infectious viruses was demonstrated by the plaque assay using the supernatant from C6/36 cell cultures infected with ZIKV at a MOI of 10 and treated or not (Mock) with 12.5, 25, 50 and 100 µg/mL of NTZ for 48h. Bars 50 µm.

Supplementary figure 3. Kinetics of DENV-2 infection and anti-DENV-2 effect of nitazoxanide on Vero cell cultures. The cultures were infected with DENV-2 at a MOI of 10 and **(A)** the detection of viral antigen (green) and nucleus of host cells (blue) were performed by immunofluorescence after 48h of infection, detecting 78.7±1.5% of infected cells. **(B)** The graph represents the means ± standard deviations of the percentage of infected cells after 24h and 48h of infection. **(C)** The infected cultures were treated or not (Mock) with 12.5, 25, 50 and 100 µg/mL of NTZ for 48h and the graph represents the means ± standard deviations of the percentage of infected cells. Except at 12.5 µg/mL of NTZ, a dose-dependent decrease in the percentage of infection was observed. The reduction of infection at 25 µg/mL of NTZ was only of 19±1%, increasing to 96.4±4.1% and 99.5±0.7%, respectively, at 50 µg/mL and 100 µg/mL. **(D)** The supernatants of infected and treated cultures were collected for the detection of infectious viruses by the plaque assay. Bars 20 µm.