Supplemental Data for

THIAZOLIDES, A NEW CLASS OF ANTI-INFLUENZA MOLECULES TARGETING VIRAL HEMAGGLUTININ AT POST-TRANSLATIONAL LEVEL

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Supplemental Figures 1, 2 and 3
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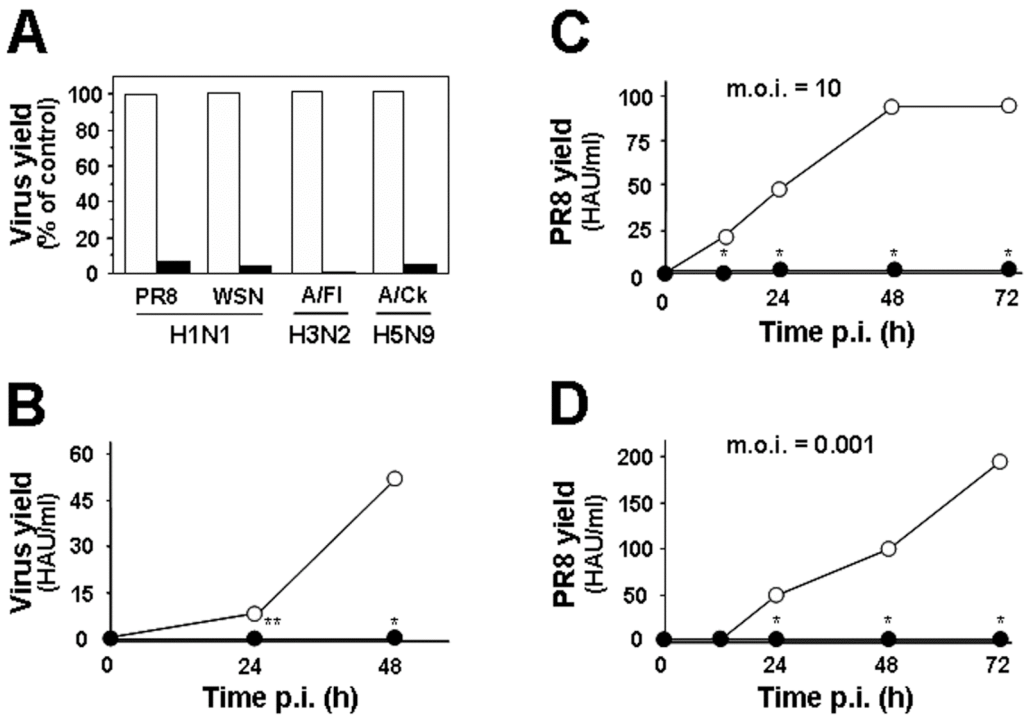
Supplemental Figure Legends

Supplemental Fig. S1. Antiviral activity of tizoxanide against influenza A and B viruses. *A*, MDCK cells were infected with four different influenza A virus strains, the mammalian H1N1 PR8 and WSN, and H3N2 A/FI, and the H5N9 avian strain A/Ck at a m.o.i. of 10 HAU/10^5 cells, and treated with 10μg/ml TIZ (*filled bars*) or vehicle (*empty bars*) immediately after the adsorption period. Virus yield was determined at 24h p.i.. *B*, long-term antiviral activity of TIZ in MDCK cells infected with influenza B virus (B/Parma/3/04) and treated with 10μg/ml TIZ (●) or vehicle (○) after virus adsorption. *C-D*, single-step (*C*) and multistep (*D*) PR8 virus growth curves were performed on MDCK cells infected at an m.o.i. of 10 (*C*) or 0.001 (*D*) ffu/cell and treated with 10μg/ml TIZ (●) or vehicle (○) as in *A*. Virus yield was determined at the indicated times p.i.. (*A-D*) Virus yield, expressed as percent of untreated control (*A*) or in HAU/ml (*B-D*) represents the mean±SD of duplicate samples from a representative experiment of three with similar results. *=P<0.01; **=P<0.05.

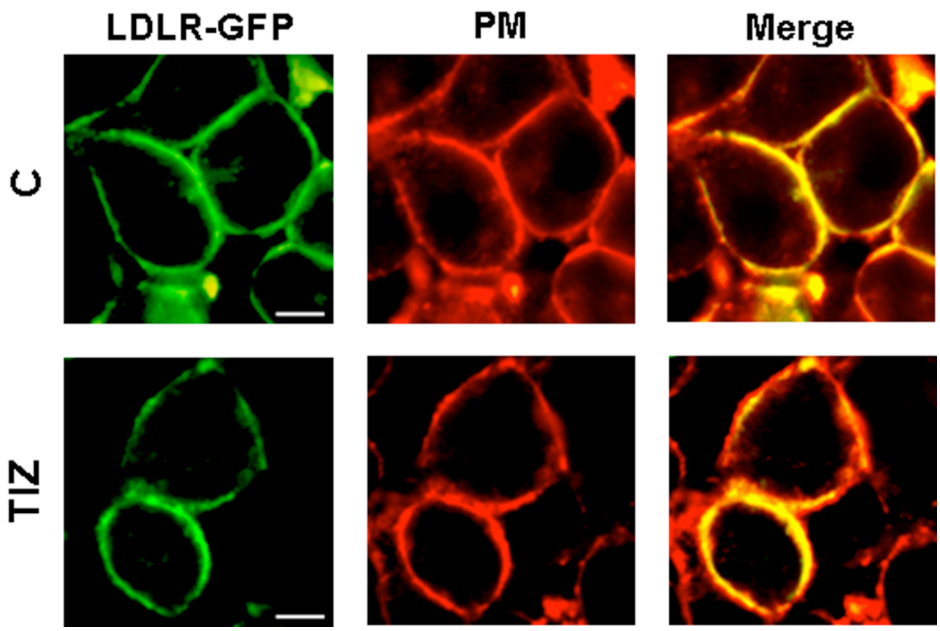
Supplemental Fig. S2. Tizoxanide does not influence human low-density lipoprotein receptor (LDLR) plasma membrane targeting. MDCK cells were transiently transfected with green fluorescent protein (GFP)-tagged internalization-defective human low-density lipoprotein receptor mutant (LDLR-A18-GFP plasmid) (40) and, after 8h, treated with TIZ (10μg/ml) or vehicle for the following 16h. After blocking protein synthesis with cycloheximide for 1h, plasma membranes were stained using CellMask™ Orange plasma membrane (PM) stain, and imaged using a Leica DM-IL fluorescence microscope equipped with UV excitation filters. The images were captured with a Leica DC-300 camera using Leica Image-Manager500 software. Levels of LDLR-GFP (*green*) and PM (*red*) were detected in untreated (*upper panels*) or TIZ-treated (*bottom panels*) transfected MDCK cells. The overlay of the two fluorochromes is shown (*merge*). Sections of the same images (bar=10μm) of a representative experiment are shown.

Supplemental Fig. S3. Effect of thiazolides on rhinovirus replication. MDCK cells infected with influenza A H1N1 PR8 virus (○), and HeLa R19 cells infected with human rhinovirus type 2 (HRV, 3 $TCID_{50}$/cell) (●) were treated with different concentrations of nitazoxanide (*A*) or tizoxanide (*B*) immediately after the adsorption period. At 24h p.i. viruses were harvested and titrated by hemagglutination assay (PR8) or infectivity assay (HRV). Data, expressed in HAU/ml (○) and $TCID_{50}$/ml (●), represent the mean±SD of duplicate samples from a representative experiment of two with similar results. *=P<0.01; **=P<0.05 *vs*. infected-control.

Supplemental Fig. S1



Supplemental Fig. S2



Supplemental Fig. S3