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Short communication

Teicoplanin inhibits Ebola pseudovirus infection in cell culture

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ABSTRACT

There is currently no approved antiviral therapy for treatment of Ebola virus disease. To discover readily available approved drugs that can be rapidly repurposed for treatment of Ebola virus infections, we screened 1280 FDA-approved drugs and identified glycopeptide antibiotic teicoplanin inhibiting Ebola pseudovirus infection by blocking virus entry in the low micromolar range. Teicoplanin could be evaluated further and incorporated into ongoing clinical studies.

Ebola virus (EBOV), a member of the *Filoviridae*, is an enveloped, filamentous, non-segmented negative-sense RNA virus that can cause deadly Ebola virus disease (EVD) (Feldmann and Geisbert, 2011). In February 2014, the largest known EVD outbreak started in Guinea, and virologic investigation identified Zaire ebola virus (ZEBOV) as the causative agent (Baize et al., 2014). As of 26 July 2015, a total of 27,784 cases and 11,294 deaths were reported (http://www.who.int/csr/disease/ebola/situation-reports/en/), resulting in a fatality rate of 40.6%. To date, no antiviral or therapeutic has been approved for treating patients with EVD, and treatment remains limited to supportive care. Therefore there is an urgent need for the discovery and development of antiviral agents against EBOV infection.

EBOV is a biosafety level 4 (BSL-4) pathogen and work with infectious EBOV is restricted to only a few BSL-4 laboratories. Hence the biology of EBOV infection remains relatively poorly understood hampering vaccine and drug development. In order to overcome this limitation, surrogate systems which allow modeling of the virus life cycle under BSL-2 conditions have been developed (Hoenen and Feldmann, 2014). Pseudoparticles expressing EBOV glycoprotein (GP) is the most commonly used tool for the study of EBOV entry and identification of EBOV entry inhibitors. Virus attachment and entry offer numerous targets for antiviral therapy and T20 (enfuvirtide), a peptide inhibitor of gp41-mediated virus entry has been successfully used in the treatment of HIV-1 infection (Altmeyer, 2004). We set out to screen approved drugs for identification of potential therapeutic options for EVD. Drug repurposing is a valid approach, and several existing drugs have been proven to be effective in the new indications (Ashburn and Thor, 2004; Chong and Sullivan, 2007).

We performed a screen of 1280 FDA-approved drugs using EBOV (Zaire strain) GP/HIV core pseudovirus containing firefly luciferase (FLuc) reporter gene (designated as pEBOV, kindly provided by Prof. Paul Zhou from Institut Pasteur of Shanghai) to identify new inhibitors. Fig. 1A shows the scheme of primary screening and hits selection process. Briefly, Vero cells (10,000 cells in 50 μl of DMEM) were seeded into each well of a white 96-well plate (Corning Costar) and incubated at 37 °C with 5% CO_2 for 24 h prior to infection. Five microliters of each test compound at a final concentration of 10 μM (diluted in assay media with a final DMSO concentration of 0.25%) were added to the plates (one compound per well). In cell control and pEBOV infection control wells, 0.25% DMSO alone was added. Within 10 min of compound addition, 45 μl

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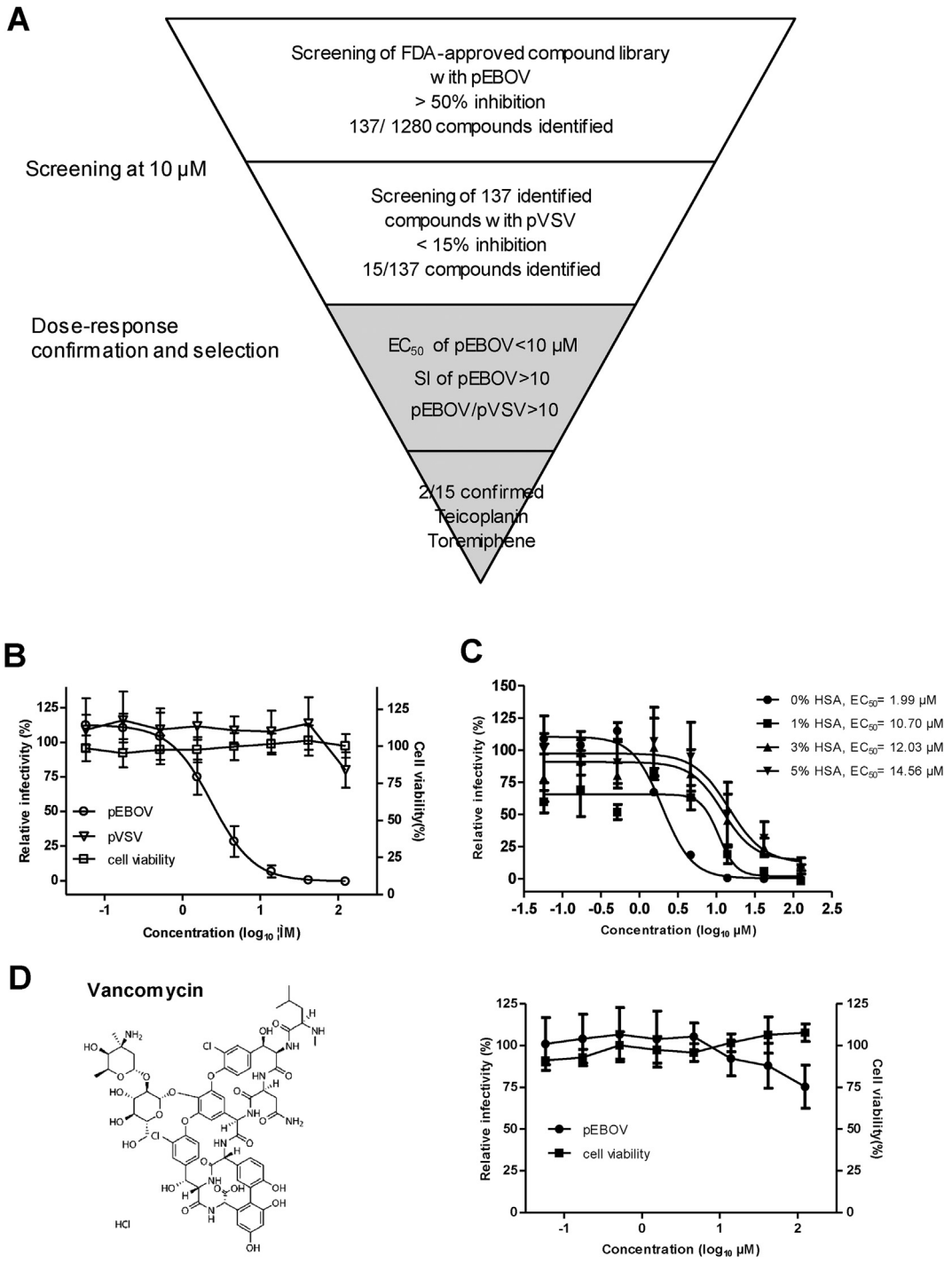

Fig. 1. Identification of teicoplanin as an inhibitor of EBOV pseudovirus (pEBOV). (A) Flowchart of screening procedure. 1280 compounds from the FDA-approved compound library were screened in single dose at 10 μM for activity against pEBOV. 137 compounds that had activity (>50% inhibition) against pEBOV were subsequently screened against VSV pseudovirus (pVSV), leading to 15 compounds that were selectively active against pEBOV. Dose-response analysis confirmed that two compounds (teicoplanin and toremiphene) met the selection criteria of EC_{50} < 10 μM, SI > 10 against pEBOV, and pEBOV/pVSV > 10. (B) Activity of teicoplanin against pEBOV and pVSV. Three-fold serial dilutions of teicoplanin were added to Vero cells, after 72 h of incubation, the relative infectivities were analyzed by measuring the luciferase and presented as a percentage of luciferase derived from the compound-treated cells compared with that from the mock-treated cells. Cytotoxicity was also examined by incubation of Vero cells with the indicated concentrations of teicoplanin and was presented as a percentage of luminescence derived from the compound-treated cells compared with that from the mock-treated cells (with medium). (C) Effect of human serum albumin (HSA) on the anti-pEBOV activity of teicoplanin. Activity of teicoplanin against pEBOV was evaluated in the presence of indicated concentrations of HSA and EC_{50}s were calculated using Prism's nonlinear regression (GraphPadPrism5). (D) Activity of vancomycin against pEBOV. Left, chemical structure of vancomycin; right, activity of vancomycin against pEBOV and cytotoxicity of vancomycin. For (B), (C), and (D), average results from three experiments are shown. Error bars represent the standard deviations (B and D) or standard error (C) of means of three independent measurements.

of 1:20-diluted pEBOV was added to each well. In cell control wells, 45 μl of assay medium was added. The final assay volume was 100 μl/well. Plates were then incubated at 37 °C with 5% CO_2 for 72 h and allowed to equilibrate to room temperature for 30 min. Afterward, 50 μl of Bright-Glo (Promega) reagent was added to each plate well, and the plates were incubated at room temperature for