Supporting Information

In vitro Anti-influenza Viral Activities of Stilbenoids from the Lianas of Gnetum pendulum

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NA activity assay

A standard fluorimetric assay was used to measure influenza virus NA activity [1]. In this assay, the substrate MUNANA is cleaved by NA to yield a fluorescent product, which can be quantified. The reaction mixture containing the test compounds and NA enzyme or a virus suspension in 32.5 mM MES buffer with 4 mM calcium chloride (pH 6.5) was incubated for 40 min at 37 °C. After incubation, the reaction was terminated by the addition of 34 mM NaOH. The fluorescence was quantified at an excitation wavelength of 360 nm and emission wavelength of 450 nm. The 50% inhibitory concentration (IC₅₀) was defined as the concentration of NA inhibitor necessary to reduce the NA activity by 50% relative to that in a reaction mixture containing virus but no...
inhibitor.

**Cytotoxicity in MDCK cells**

Cell viability was determined with the MTT method [2]. Consecutive threefold serial dilutions (100 μL) of the stilbenoids from the lianas of *G. pendulum* and the reference compounds, oseltamivir acid and ribavirin, were added to cell monolayers in triplicate. Blank medium was used as control. After three days of incubation at 37°C, 12 μL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well. The plate was further incubated at 37 °C for 3 h to allow the formation of the formazan product. After removal of the medium, 100 μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. After 15 min, the contents of the wells were homogenized on a microplate shaker. The optical densities were then measured with a microplate spectrophotometer at a wavelength of 540 nm. The median cytotoxic concentration (CC\textsubscript{50}) was calculated as the concentration of the constituent that reduced the number of viable cells to 50% of the untreated control. The maximal non-cytotoxic concentration (MNCC) was defined as the maximal concentration of the sample that did not exert a cytotoxic effect and resulted in more than 90% viable cells.

**CPE reduction assay**

The antiviral activities of the stilbenoids isolated from the lianas of *G. pendulum* were measured with the CPE inhibition assay [3]. Viral suspension (200 TCID\textsubscript{50}/mL, 100 μL) was added to each well of a 96-well plate containing a confluent cell monolayer. After incubation at 37 °C for 2 h, the virus solution was removed, and 100 μL of consecutive threefold serial dilutions of the test constituents and reference compounds were added to each well, using the MNCC as the highest concentration. An infection control without constituents was also included. The plates were incubated at 37 °C in a humidified CO\textsubscript{2}
atmosphere (5% CO₂) for 24 h, after which the CPE was assessed. The virus-induced CPE was scored as follows: 0 = no CPE, 1 = 0–25% CPE, 2 = 25%–50% CPE, 3 = 50%–75% CPE, and 4 = 75%–100% CPE. The reduction in virus multiplication was calculated as a percentage of the virus control (% virus control = CPE_{exp} / CPE_{virus control} × 100). The IC₅₀ of the CPE with respect to the virus control was estimated using the Reed–Muench method and is expressed in μg/mL. The selective index (SI) was calculated as the ratio CC₅₀/IC₅₀.

Reagents

2′-(4-Methylumbelliferyl)-α-D-acetyleneuraminic acid (MUNANA), 2-N-morpholino-ethanesulfonic acid (MES), Dulbecco’s minimum essential medium (DMEM), and 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich, Inc.. Trypsin–EDTA and trypsin (1:250) were from Gibco Industries. Fetal bovine serum (FBS) was from Biofluids Inc.. Oseltamivir carboxylic acid (98% purity) was purchased from Toronto Research Chemicals Inc., and ribavirin (98% purity) was provided by the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences.

References
