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A Colorimetric Cell Culture Assay for the Identification of SARS Coronavirus Inhibitors

E. Keyaerts[1], L. Vijgen[1], J. Neyts[1], E. De Clercq[1], J. Balzarini[1], M. Van Ranst[1,2]

[1]Rega Institute for Medical Research, K.U. Leuven, 3000 Leuven, Belgium; [2]U.Z. Leuven, 3000 Leuven, Belgium

Severe acute respiratory syndrome (SARS) has recently emerged as a new severe human disease, resulting globally in 774 deaths from 8098 reported probable cases. A novel member of the *Coronaviridae* family has been identified as the causative agent of this pulmonary disease. Although the initial global outbreak of SARS appears to have been successfully contained, SARS will remain a serious concern while there continues to be no suitable vaccine or effective drug treatment. A colorimetric assay based on the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) into a water soluble formazan product which can be directly quantified using a microtiter ELISA reader, has been developed for SARS coronavirus strain Frankfurt 1 drugsusceptibility testing. Optimal conditions were determined and the standard routine assay was calibrated with a viral input of 100 $CCID_{50}$ and a density of 10,000 cells per well in a 96-well microtiter plate for an incubation period of 3 days. Interferon β was used as a positive control to validate the assay. The effective IC_{50} concentration value obtained with interferon β in the present assay was in agreement with interferon β activity results published by others. This method presents the advantage of being rapid, reliable, reproducible, and convenient for high throughput screening capacity in a stringent P3 biosafety environment.

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Antiviral Activity of Glycyrrhizic Acid (GL) Derivatives Against SARS-Coronavirus (SARS-CoV) and Human Cytomegalovirus (HCMV)

G. Hoever[1], L. Baltina[2], R. Kondratenko[2], L. Baltina Jr.[2], G. Tolstikov[2], H.W. Doerr[1], J. Cinatl[1]

[1]Institute of Medical Virology, Johann Wolfgang Goethe University Frankfurt, Paul-Ehrlich Str. 40,60596 Frankfurt, Germany; [2]Institute of Organic Chemistry Ufa Research Centre of Russian Academy of Sciences, Prospect Oktyabrya, 71, Ufa

Glycyrrhizic Acid (GL) is the major bioactive triterpene glycoside of licorice root (Glycyrrhiza Radix). The antiviral activity of GL against a broad spectrum of viruses, among others HIV, HSV1, Influenza Virus, SARS-CoV, HBV and HCV has been reported.

In the effort to discover GL analogous with strong enhanced antiviral activity, we tested a number of new synthesized GL derivatives against HCMV and SARS-CoV. These compounds were received by introduction of different functional groups in the Carboxyl and Hydroxyl groups as well as transformations of the carbohydrate part of the molecule.

Our results show that the GL-amides BL43 and BL49, the reduced GL-trimethyl ester BL 44, and the Glycopeptide L-Cys-GL present a more than 10-fold increased antiviral activity against SARS-CoV compared to Glycyrrhizin, while the GL-amides BL26, and BL43 presented antiretroviral activity against HCMV.

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Antiviral and Virucidal Activities of Oreganol P73-based Spice Extracts Against Human Coronavirus In Vitro

M.K. Ijaz[1,2,3], Z. Chen[1], S.S. Raja[1], D.B. Suchmann[1], P.W. Royt[2], C. Ingram[4], J.K. Gray[4], G. Paolilli[4]

[1]MICROBIOTEST, INC., Sterling, VA 20164, USA; [2]George Mason University, Fairfax, VA 22030, USA; [3]University of Ottawa, Ottawa, Ont., Canada; [4]North American Herb & Spice, Buffalo Grove, IL 60085, USA

Human Coronavirus (HCoV) infection is very common, disseminated by air and occurs worldwide. Recently, a previously unknown HCoV has been implicated as a causative agent of severe acute respiratory syndrome (SARS). Finding a successful antiviral drug for SARS-associated HCoV is particularly challenging. The virucidal and antiviral activities of two Oreganol P73-based spice extracts were evaluated during in vitro HCoV (ATCC VR-740) infection. To determine, the virucidal potentials of non-cytotoxic dilutions of Oreganol P73 Extra Strength Formula (0.1%) and Oregacyn (0.01%), the virus was exposed to each drug dilution, and samples were collected at various times post-exposure prior to assay on the host cells. The antiviral ability of both Oreganol P73 Extra Strength Formula and Oregacyn was determined by maintaining the non-cytotoxic concentration of both drugs throughout the viral–host interaction period. The cell culture plates were examined microscopically for the presence of HCoV-induced cytopathic effects produced by viral infection. The virucidal part of the study indicated that both Oreganol P73 Extra Strength Formula and Oregacyn at final concentrations of 0.1 and 0.01%, respectively, proved to be coronavirucidal in direct proportion to exposure time ranging from 2 to 20 min at ambient temperature. In contrast, the antiviral studies revealed both Oreganol P73 Extra Strength Formula and Oregacyn completely inhibited HCoV infection in vitro. These data indicate the potential value of these Oreganol P73-based spice extracts as anti-HCoV compounds and merit further investigation against other mammalian viruses including HIV, HBV, HCV, influenza-, parainfluenza-, respiratory syncytial virus, herpes-, Hanta- and West Nile viruses.