remedies in models of experimental arboviral infections: Tick-borne encephalitis (TBE) and West Nile fever (WNF). Phytoremedy SK-19 was introducing three times with 3 days intervals in doses of 20–50 mg/kg. This treatment and prevention scheme caused resistance of mice to TBE virus within the limits of 24.0–29.3% of protection. Similarly, individual introduction of E-ACA in doses of 1000–2000 mg/kg to infected mice showed low activity (8% of protection). At the same time, the combined application of the above-mentioned preparations using the same schemes provided protection of 35.1–43.8% of infected mice, increasing their survival by 11.1–14.5% compared with the introduction of individual SK-19. A similar pattern was observing when we used amixin and E-ACA in experimental WNF. In this case amixin in an optimal three times injection in dose of 150 mg/kg prevented death of 56.1% of mice, and E-ACA, respectively – 11.2% of mice. Analogous combined use of the above-mentioned preparations was accompanied by appreciable increase of protective action which was characterized by the protection of 67.7–71.5% of animals. It was more by 11.6–15.4% in comparison with an individual introduction of amixin. Also, a significant increase of average duration of life of the animals (by 3.6–4.1 days for TBE and by 5.1–5.6 days for WNF) compared to benchmarks shows the reliable effectiveness of the proposed method of prevention and treatment of TBE and WNF.

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HIV Full-Replication Technology for Identification of Novel HIV Inhibitors from Multiple Sources

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Control of the global HIV/AIDS health threat depends on a continuously flowing pipeline of novel anti-HIV agents for the development of new anti-HIV therapies. HIV with its multiple target-sites and the potential to evade current antiretroviral therapy (HAART) requires a constant and accelerated search for novel therapeutics. Here we report the establishment of a technically streamlined, sensitive and robust cell-based assay system (EASY-HIT) for the unbiased identification and characterization of HIV inhibitors. The assay allows discrimination between inhibitors of early and late phases of HIV replication and yielded high Z' scores (>0.9) and signal stabilities, confirming its robustness. Application of EASY-HIT to screening of various compound libraries identified several novel HIV-inhibitory molecules which are currently under further evaluation as potential lead candidates. Furthermore, we demonstrate the successful application of the assay for the detection of anti-HIV activities in crude biological extracts. Natural products with their largely unexplored diversity present a promising source for the discovery of novel anti-HIV drugs. Extracts derived from terrestrial plants as well as marine macroalgae showed strong activities against various targets of the viral replication cycle such as attachment, viron fusion and provirus integration. Several follow-up assays were applied for the characterization of inhibitory activities with a strong focus on viral entry as well as Tat- and Rev-dependent HIV gene expression.

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An Antiviral Assay to Identify Inhibitors of the Human Metapneumovirus that is Amenable for High-throughput Screening Purposes

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Human metapneumovirus (hMPV) is a recently discovered RNA virus in the pneumovirus subfamily of the Paramyxoviridae. Similar to RSV, it is an important cause of respiratory tract infections in infants, young children, and in immunocompromised individuals. Specific antiviral therapy is not available. Here we describe the setup of an antiviral assay against hMPV that is suitable for high-throughput screening purposes. To this end GFP-expressing recombinant hMPV NL/1/00 (De Graaf et al., 2007, J Virol Methods) was employed. Vero118 cells were infected with this recombinant virus (MOI = 0.01, 200 μL cell culture, 2×10^4 cells/mL, IMDM medium) after which cultures were further incubated for 4 days at 37 °C. Fluorescence was quantified using a Saphire^2 system. This method proved to be suitable for high-throughput screening it combined a homogenous assay set-up with good assay quality (Z': 0.5–0.6). Ribavirin was used as a positive control and allowed to validate the assay for antiviral screening purposes. In conclusion an antiviral assay was successfully optimized to a format that allows high-throughput screening for inhibitors of hMPV replication.

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PHYTOCHIK: Biodiversity As A Source of Selective Inhibitors of CHIKV Replication

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Chikungunya virus (CHIKV) is an alphavirus that is transmitted to humans mainly by the Aedes aegypti or albopictus mosquito. Ever since the outbreak on Reunion Islands in 2005–2006, CHIKV is considered to be an emerging virus. In particular arthralgia or arthritis, affecting multiple joints, is very pronounced during the clinical course, and may last for as long as 2 years. Currently, no vaccine nor a selective antiviral drug is available for the prevention or treatment of this debilitating viral infection. Chloroquine is active in cell culture and may alleviate the symptoms of arthritis by acting as an anti-inflammatory agent, although this latter is still under investigation. In 2008, the CRVOI (Centre de Recherche et de Veille sur les maladies émergentes dans l'Océan Indien)