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Identification and evaluation of antiviral compounds targeting Rift Valley fever virus

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Abstract

Rift Valley fever virus (RVFV), a negative-stranded RNA virus, is the etiological agent of the vector-borne zoonotic disease Rift Valley fever (RVF). RVFV causes significant morbidity and mortality in humans and livestock throughout Africa and Arabian Peninsula. RVFV is an emerging virus and is capable of infecting a broad range of mosquito species distributed around the world, therefore it poses a potential threat globally. A wide range of livestock animals (e.g. sheep, goat, cow, camel) and some wild animals become highly affected by RVFV. In humans RVFV infection presents as an acute self-limiting febrile illness that may lead to a more severe hemorrhagic fever and encephalitis. Severity of the disease is mostly dependent on age and mammalian species together with other factors.

There is an absence of licensed RVFV vaccines for humans, and lack of effective antiviral drugs. Moreover, due to the severe pathogenicity, higher level facilities are needed, bio-safety level 3 (BSL-3) or more, to work with RVFV, which makes antiviral drug development more challenging. Because RVFV cause severe disease in Africa and the Arabian Peninsula, and has the potential to spread globally, it is essential to develop safe efficient antivirals for this virus.

The previously reported antiviral compound benzavir-2 inhibits several DNA viruses, i.e. human adenovirus, herpes simplex virus type 1, and type 2 infection, indicating a broad-acting activity. We wanted to evaluate whether benzavir-2 had an effect against the RNA virus RVFV. For these and subsequent studies, we used a recombinant, modified RVFV with a deleted NSs gene, replaced by a reporter gene, which enabled the studies to be conducted in BSL-2 conditions. The NSs gene is the main virulence factor for RVFV and in absence of it, RVFV become less pathogenic. The reporter gene made it possible to detect red fluorescent protein after virus infection and use it to quantify infection. We observed that benzavir-2 effectively inhibited RVFV infection in cell culture with an effective concentration showing 50% inhibition (EC50) at 0.6 μ M. Benzavir-2 also inhibited the production of progeny viruses. When we studied the pharmacokinetics properties, we found that benzavir-2 had good *in vitro* solubility, permeability and metabolic stability. When we investigated the oral bioavailability in mice by administering benzavir-2 in peanut butter pellets, high systemic distribution was observed without adverse toxic effects. Benzavir-2 thus inhibited RVFV infection in cell culture and demonstrated excellent pharmacokinetic properties with the potential to evaluate the effectiveness in an animal model. Since benzavir-2 has a broad effect against both RNA and DNA viruses, we speculate that the antiviral mechanism affects cellular targets.

We also wanted to explore a large number of small chemical compounds of unknown properties and identify anti-RVFV activities. Therefore, we developed a whole-cell-based high-throughput screening assay based on the recombinant, modified RVFV with a deleted NSs gene, replaced by a reporter gene, and screened 28,437 small chemical compounds. The assay was established after optimization of several parameters. After primary and secondary screening, we identified 63 compounds that inhibited RVFV infection by 60% at 3.12 μ M and showed \geq 50% cell viability at 25 μ M concentration. After a dose dependent screening of these 63 compounds several compounds were identified with highly efficient anti-RVFV properties. Finally N¹-(2-(biphenyl-4-yl)oxy)ethyl)propane-1,3-diamine (compound 1) was selected as lead compound. We performed a structure activity relationship analysis (SAR) of compound 1 by replacing and changing component after component of the chemical compound to see how it affected the antiviral activity. After the SAR analysis, the antiviral activity did not change, however we could improve the cytotoxicity profile. The improved compound was named 13a, and our study suggested that compound 13a might be active during the early phase of RVFV lifecycle.

To sum up, we developed an efficient and reliable screening method that opens up possibilities to discover and develop antivirals against RVFV in BSL-2 condition. We also identified several chemical compounds with anti-RVFV activities, which might lead to development of therapies against RVFV.

Keywords

Rift Valley fever virus, antiviral, assay development, screening, drug discovery.

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