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Alphavirus protease inhibitors from natural sources: A homology modeling and molecular docking investigation

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ABSTRACT

Alphaviruses such as Chikungunya virus (CHIKV), O'Nyong–Nyong virus (ONNV), Ross River virus (RRV), Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), and Western equine encephalitis virus (WEEV), are mosquito-transmitted viruses that can cause fevers, rash, and rheumatic diseases (CHIKV, ONNV, RRV) or potentially fatal encephalitis (EEEV, VEEV, WEEV) in humans. These diseases are considered neglected tropical diseases for which there are no current antiviral therapies or vaccines available. The alphavirus non-structural protein 2 (nsP2) contains a papain-like protease, which is considered to be a promising target for antiviral drug discovery. In this work, molecular docking analyses have been carried out on a library of 2174 plant-derived natural products (290 alkaloids, 664 terpenoids, 1060 polyphenolics, and 160 miscellaneous phytochemicals) with the nsP2 proteases of CHIKV, ONNV, RRV, EEEV, VEEV, WEEV, as well as Aura virus (AURV), Barmah Forest Virus (BFV), Semliki Forest virus (SFV), and Sindbis virus (SINV) in order to identity structural scaffolds for inhibitor design or discovery. Of the 2174 phytochemicals examined, a total of 127 showed promising docking affinities and poses to one or more of the nsP2 proteases, and this knowledge can be used to guide experimental investigation of potential inhibitors.

1. Introduction

Alphaviruses are a genus of arthropod-borne viruses (arboviruses) that belong to the family *Togaviridae*. They are commonly referred to as "Old World" and "New World" viruses depending on the geographical location where they were originally isolated. The "Old World" alphaviruses include Chikungunya virus (CHIKV), O'Nyong–Nyong virus (ONNV), Ross River virus (RRV), Semliki Forest virus (SFV), and Sindbis virus (SINV). Infection by "Old World" alphaviruses is generally associated with fever, rash, and rheumatic diseases such as polyarthralgia or polyarthritis. Although these infections can be chronic and debilitating, they are rarely fatal. 'New World' alphaviruses include Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV) and Western equine encephalitis virus (WEEV). These viral infections are primarily associated with potentially fatal encephalitis in humans and other mammals. These viral infections, usually transmitted by mosquitoes, can be considered neglected diseases. For recent reviews on alphaviruses, see (Gould et al., 2010; Leung et al., 2011; Suhrbier et al., 2012).

Chikungunya virus (CHIKV) was initially recognized as a human pathogen in 1952 in Tanzania (formerly Tanganyika). The virus typically causes fever, skin rash, and debilitating arthralgia (Pialoux et al., 2007). This tropical disease is mainly spread by two species of *Aedes* mosquito, *A. albopictus* and *A. aegypti*. Many cases of chikungunya fever have been identified in Africa, Asia, and more recently in Europe and the Western Hemisphere (Weaver, 2014; Morens and Fauci, 2014; Rougeron et al., 2015). A large epidemic of CHIKV fever occurred in 2004, originating in Kenya and spreading throughout islands in the Indian Ocean (Staples et al., 2009). In 2013–2014 a chikungunya outbreak occurred in the Caribbean region (Van Bortel et al., 2014), and the virus has now been found in northern South America, Central America, and southeastern United States, accounting for more than 1 million CHIKV infections (Staples and Fischer, 2014).

O'Nyong–Nyong virus (ONNV) is closely related to CHIKV (Powers et al., 2000). This virus caused major epidemic outbreaks in East Africa in 1959, southern Uganda in 1996 (Kiwanuka et al., 1999), and has appeared in the Ivory Coast in 2003 (Posey et al., 2005) and Chad in 2004 (Bessaud et al., 2006). ONNV is typically transmitted by anopheline mosquitoes, *Anopheles funestus* and

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Anopheles gambiae, but has also been isolated from a culicine mosquito, *Mansonia uniformis* (Lutwama et al., 1999). The clinical manifestations of ONNV infection are similar to CHIKV infection and typically involve fever, maculopapular skin rash, pruritus, myalgias, and arthralgias (Kiwanuka et al., 1999).

Ross River virus (RRV) is endemic to Australia and Papua New Guinea, but outbreaks have occurred in the South Pacific (Harley et al., 2001; Jacups et al., 2008). The virus was first isolated in 1959 from *Aedes vigilax* mosquitoes trapped along the Ross River in Townsville, north Queensland. RRV spread into the South Pacific in 1979 causing large epidemics in New Caledonia, Fiji, Samoa, and the Cook Islands. RRV infection most often causes rash, fever, myalgia, and arthralgia, and sometimes arthritis. The major mosquito vectors of RRV are *Aedes vigilax*, *Aedes camptorhychus*, and *Culex annulifostris*. A related, but less common alphavirus disease in Australia is Barmah Forest virus (BFV) disease (Quinn et al., 2005, 42, 882–890). Symptoms of BFV infection are similar to those of RRV (Jacups et al., 2008).

Semliki Forest virus (SFV), first isolated from *Aedes abnormalis* mosquitoes in the Semliki Forest of Uganda (Manwaring, 1945), is naturally found in sub-Saharan Africa and is principally spread by *Aedes africanus* and *Aedes aegypti* mosquitoes (Fazakerley, 2002). SFV infection in humans generally results in mild fever or can be asymptomatic, but virulent strains of the virus cause lethal encephalitis in mice (Atkins et al., 1999). An outbreak of SFV infection did occur in the Central African Republic in 1987 and several patients presented fever, severe and persistent headache, myalgia, and arthralgia (Mathiot et al., 1990).

Sindbis virus (SINV) was first isolated from *Culex pipiens* and *Culex univittatus* mosquitoes from the village of Sindbis, Egypt (Kurkela et al., 2008). The virus is the causative agent of Pogosta disease in Finland, Ockelbo disease in Sweden, and Karelian fever in Russia and is transmitted by *Culex* spp. and *Culiseta* spp. mosquitoes (Kurkela et al., 2004). SINV is widespread in the Old World, including northern Europe, South Africa, Australia, and China. SINV fever in humans can cause mild fever, itching rash, fatigue, myalgia, and arthralgia.

Venezuelan equine encephalitis virus (VEEV) is a “New World” arbovirus that causes Venezuelan equine encephalitis (Weaver et al., 2004). VEEV is restricted to tropical and subtropical Americas, from Argentina to south Texas. There are at least 13 distinct subtypes and varieties that make up the VEEV complex, of which only types IAB and 1C have caused major outbreaks (Aguilar et al., 2004). A major outbreak occurred in 1995 in La Guajira, Columbia, attributed to serotype IC and spread principally by the *Aedes taeniorhynchus* mosquito vector (Rivas et al., 1997). The outbreak caused an estimated 75,000 human cases with 3000 neurological complications and 300 deaths. Humans with serotype IC infection had acute, self-limited fever, but convulsions were often seen in children, and abortions and fetal deaths occurred in pregnant women (Weaver et al., 1996). In 1969–1972 there was a major outbreak of VEEV serovar IAB on the Guatemala-El Salvador border that spread northward through Mexico and into southern Texas, and caused around 50,000 equine deaths and 93 confirmed human deaths in Mexico (Estrada-Franco et al., 2004). Endemic VEEV serovar ID has been the cause of at least 3% of the febrile diseases in Iquitos, Peru (Watts et al., 1998). In humans, serotype ID infection causes acute, undifferentiated fever, headache, malaise, and arthralgia (Oberste et al., 1998; Aguilar et al., 2004). VEEV is spread by mosquitoes, principally *Culex* spp. (Aguilar et al., 2004), *Aedes* spp. and *Psorophora* spp. (Alfonzo et al., 2005).

Eastern equine encephalitis virus (EEEV) is closely related to VEEV (Zacks and Paessler, 2010). The disease ranges from Eastern North America, through Central America, the Caribbean, and into South America as far south as Argentina (Aguilar et al., 2007). The disease is rare in humans with 82 confirmed cases of EEEV infection in the U.S. from 2004 to 2013 (Centers for Disease Control and Prevention, 2016), most of which occur in the Atlantic and Gulf States. Nevertheless, the disease is virulent with a mortality rate in humans around 33% and significant brain damage in most survivors. The enzootic transmission cycle is between birds and ornithophilic mosquitoes (*Culiseta melanura* in North America) (Armstrong et al., 2008), but *Aedes* spp., *Coquillettidia* spp., and *Culex* spp. are likely bridge vectors for transmission of EEEV to mammals in North America (Centers for Disease Control and Prevention, 2016).

Western equine encephalitis virus (WEEV), similar to VEEV and EEEV, is found in western North America and South America (Forrester et al., 2008). Similar to EEEV, the virus is maintained with a mosquito-bird transmission cycle with *Culex tarsalis* as the primary vector in western North America. Transmission to equines and humans is facilitated by bridging mosquito vectors, *Ochlerotatus melanimon* in California, *Aedes dorsalis* in Utah and New Mexico, and *Aedes campestris* in New Mexico (Zacks and Paessler, 2010). WEEV infection is often asymptomatic, but the disease can cause severe symptoms (fever, headache, nausea, malaise) in infants and the elderly with a mortality rate of around 4% (Zacks and Paessler, 2010).

Aura virus (AURV) is closely related to the “Old World” Sindbis virus (SINV) (Rümenapf et al., 1995). The virus is found in Brazil and Argentina and has been isolated from the *Aedes serratus* mosquito. The virus apparently does not cause disease in humans (Zhang et al., 2002).

Viral replication in alphaviruses depends on four non-structural proteins, nsP1-nsP4, which are produced as a single polyprotein (Gould et al., 2010). Proteolytic processing of the polyprotein by the protease in nsP2 gives the four individual non-structural proteins. The nsP1 carries RNA methyltransferase activity. The nsP2 has two domains, a helicase/nucleotide triphosphatase domain in the N-terminal region and a papain-like cysteine protease domain in the C-terminal region. The nsP3 has a macro domain with binding affinity for ADP-ribose and diphosphate-ribose 1″-phosphate activity. The nsP4 contains the viral RNA-dependent RNA polymerase. The nsP2 protease domain, responsible for non-structural polyprotein processing and essential for virus replication, is considered to be a promising target for antiviral drug discovery (Russo et al., 2010).

Most alphavirus diseases are considered to be neglected tropical diseases afflicting people living in the poorest conditions, and these people generally rely on naturopathy for their medical needs (Gubler, 2002; Kline et al., 2013; Rougeron et al., 2015). There are currently no licensed human vaccines against alphaviruses and there are currently no effective antiviral therapeutic agents available (Gould et al., 2010). Current treatment has been based on treating symptoms with analgesics and non-steroidal anti-inflammatory agents. It is important to identify potential phytochemical agents that can be used to treat alphavirus infections, serve as inexpensive and readily available treatment, and may also serve as templates for development of efficacious antiviral agents. In this work, we have carried out an *in-silico* screening of compounds from our virtual library of phytochemicals against alphavirus nsP2 protease structures using a molecular docking approach.

2. Computational methods

Protein-ligand docking studies were carried out based on the crystal structures of chikungunya virus (CHIKV) nsP2 protease (PDB 3TRK) (Cheung et al., 2016) and Venezuelan equine encephalitis virus (VEEV) nsP2 protease (PDB 2HWK) (Russo et al., 2006), and homology-modeled structures of Aura virus