Antiviral Research 144 (2017) 216–222

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Antiviral activity of [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones against chikungunya virus targeting the viral capping nsP1

Alba Gigante [a, 1], Asier Gómez-SanJuan [a, 1], Leen Delang [b], Changqing Li [c], Oskía Bueno [a], Ana-María Gamo [a], Eva-María Priego [a], María-José Camarasa [a], Dirk Jochmans [b], Pieter Leyssen [b], Etienne Decroly [c], Bruno Coutard [c], Gilles Querat [d], Johan Neyts [b], María-Jesús Pérez-Pérez [a, *]

[a] Instituto de Química Médica (IQM-CSIC), Juan de la Cierva 3, E-28006, Madrid, Spain
[b] KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, B-3000, Leuven, Belgium
[c] Aix Marseille Univ, CNRS, AFMB UMR7257, Marseille, France
[d] UMR "Émergence des Pathologies Virales" (EPV: Aix-Marseille Univ – IRD 190 – Inserm 1207 – EHESP – IHU Méditerranée Infection), Marseille, France

ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form
27 April 2017
Accepted 8 June 2017
Available online 12 June 2017

Keywords:
Triazolopyrimidines
Oximes
Chikungunya virus
Alphavirus
mRNA capping

ABSTRACT

Chikungunya virus (CHIKV) is a re-emerging alphavirus transmitted to humans by *Aedes* mosquitoes. Since 2005, CHIKV has been spreading worldwide resulting in epidemics in Africa, the Indian Ocean islands, Asia and more recently in the Americas. CHIKV is thus considered as a global health concern. There is no specific vaccine or drug available for the treatment of this incapacitating viral infection. We previously identified 3-aryl-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones as selective inhibitors of CHIKV replication and proposed the viral capping enzyme nsP1 as a target. This work describes the synthesis of novel series of related compounds carrying at the aryl moiety a methylketone and related oximes combined with an ethyl or an ethyl-mimic at 5-position of the triazolopyrimidinone. These compounds have shown antiviral activity against different CHIKV isolates in the very low μM range based on both virus yield reduction and virus-induced cell-killing inhibition assays. Moreover, these antivirals inhibit the *in vitro* guanylylation of alphavirus nsP1, as determined by Western blot using an anti-cap antibody. Thus, the data obtained seem to indicate that the anti-CHIKV activity might be related to the inhibition of this crucial step in the viral RNA capping machinery.

1. Introduction

Chikungunya virus (CHIKV) is an alphavirus that is transmitted to humans by *Aedes* mosquitoes, traditionally *Aedes aegypti* and more recently also *Aedes albopictus*. CHIKV causes chikungunya fever, a disease that is characterized by fever, nausea, headaches, rash and a persistent arthralgia (Burt et al., 2012; Thiberville et al., 2013). Although the clinical course is rarely associated with a fatal outcome, the symptoms can be severe and disabling, and may last for a long period of time (Couderc and Lecuit, 2015). In neonates, elderly people or patients with underlying medical conditions such as diabetes or heart disease, chikungunya fever can be associated with severe complications, including death (Couderc and Lecuit, 2015; Thiberville et al., 2013). Of particular concern are patients with a pre-existing arthritic disease that become infected by CHIKV (Burt et al., 2014).

Since first reported in 1952, CHIKV has been the cause of sporadic and infrequent outbreaks in Africa and Asia. In the last 10 years, the situation has dramatically changed and CHIKV is now considered a re-emerging virus that is a global health threat (Powers, 2015; Rougeron et al., 2015; Thiboutot et al., 2010). From 2004 onwards, CHIKV outbreaks have resulted in millions of cases reported on the five continents. Phylogenetic tools are being used to reconstruct the geographic spread of CHIKV outbreaks and to characterize the circulating virus, a crucial issue for the prediction and control of the CHIKV outbreak (Lo Presti et al., 2016; Weaver and Forrester, 2015). As an example, since December 2013, with the first reported case in the Caribbean island of Saint Martin, the

* Corresponding author.
E-mail address: mjperez@iqm.csic.es (M.-J. Pérez-Pérez).
[1] Both authors have equally contributed to this work.

http://dx.doi.org/10.1016/j.antiviral.2017.06.003

virus has expanded to more than 20 countries in the Americas. Mediterranean Europe is also considered a potential area for CHIKV expansion, and autochthonous cases have already been reported in Italy and France (Delisle et al., 2015). Moreover, CHIKV infections are considered to be underestimated since the clinical symptoms and geographic distribution partially overlaps with dengue virus infection (Lo Presti et al., 2016).

There are no specific drugs to prevent or cure CHIKV infections (Abdelnabi et al., 2015; Ahola et al., 2015; Kaur and Chu, 2013; Rashad et al., 2013). Treatment is primarily directed at relieving the symptoms with over-the-counter drugs (Thiberville et al., 2013). Taking the severe impact of this infection into account, there is yet an unmet need for the discovery of compounds that are able to effectively and selectively interfere with the replication of CHIKV (Abdelnabi et al., 2015; Kaur and Chu, 2013). As the likelihood to develop chronic CHIKV disease seems to be correlated with the severity of symptoms during the acute phase of infection, a potent antiviral administered during the acute infection may diminish the chances to develop chronic disease (Abdelnabi et al., 2017). However, it is not clear yet whether anti-CHIKV therapy may be beneficial on the development of chronic CHIKV-induced arthritis. Compounds from natural origin such as flavonoids (Lani et al., 2016), known drugs such as niclosamide (Wang et al., 2016) and suramine (Albulescu et al., 2015; Kuo et al., 2016) or synthetic compounds (Ching et al., 2015; Mishra et al., 2016) have been recently reported to inhibit CHIKV infection in cell-based assays. Moreover, the alkaloid berberine has been shown to inhibit MAPK signaling activated by CHIKV infection, leading to antiviral effects in an animal model (Varghese et al., 2016).

In 2014, we discovered a series of small molecules that selectively block CHIKV replication. We identified compound **1** (Fig. 1) as an initial hit (Gigante et al., 2014). Structure-activity relationship studies revealed that the [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one structure and the *meta*-substituted aryl ring linked at position 3 of the triazole were critical for anti-CHIKV activity. Accordingly, a significant improvement in terms of antiviral activity was observed for compound **2**, which has an ethyl substituent at position 5 of the heterocyclic base (Fig. 1). In addition, we recently showed that under the antiviral pressure of compound **1** drug resistant CHIKV strains were selected that carried a P34S substitution in the non-structural protein (nsP1). The importance of this mutation for the resistance phenotype was confirmed by reverse genetics (Delang et al., 2016), demonstrating that the nonstructural protein 1 (nsP1) carrying the function for the mRNA capping is targeted by our hit compound **1**. Indeed, compound **1** inhibited the guanylyltransfer (GT) activity of the nsP1 of Venezuelan equine encephalitis virus (VEEV) that was used as the enzymatic model for the study (Delang et al., 2016). In this study, we have now synthesized new derivatives analogous to **1** carrying at the aryl moiety a methylketone and related oximes combined with an ethyl or an ethylmimic at 5-position of the triazolopyrimidinone (Fig. 1). We have determined their antiviral activity against CHIKV in virus-cell-based assays, and the inhibition of the GT transfer by VEEV nsP1 for the most potent compounds.

2. Materials and methods

2.1. Chemistry procedures

Melting points were obtained on a Mettler Toledo M170 apparatus and are uncorrected. The elemental analysis was performed with a Heraus CHN-O-RAPID instrument. The elemental compositions of the compounds agreed to within ±0.4 of the calculated values. For all the tested compounds, satisfactory elemental analysis was obtained supporting greater than 95% purity. Electrospray

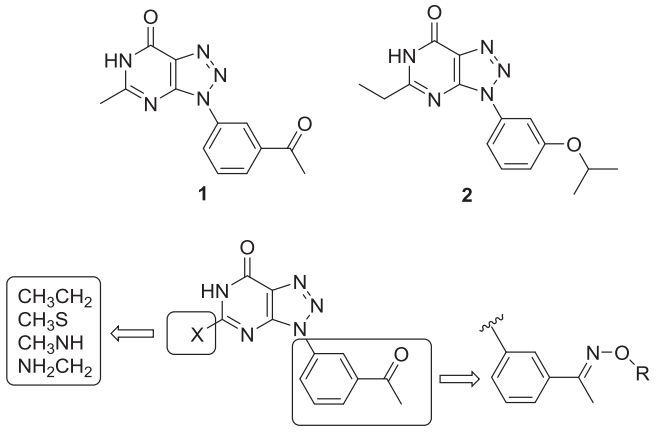

Fig. 1. Previously identified inhibitors of CHIKV replication based on [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-ones and general structures of the compounds addressed herein.

mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MS HP 1100). ^{1}H and ^{13}C NMR spectra were recorded on a Varian INNOVA-300 operating at 300 MHz (^{1}H) and 75 MHz (^{13}C), respectively, and a Varian INNOVA-400 operating at 400 MHz (^{1}H) and 101 MHz (^{13}C), respectively.

Analytical TLC was performed on silica gel 60 F_{254} (Merck) precoated plates (0.2 mm). Spots were detected under UV light (254 nm) and/or charring with ninhydrin. Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron^{R} (Kiesegel 60 F_{254} gipshaltig (MercK)), with layer thicknesses of 1 and 2 mm and flow rates of 4 or 8 mL/min, respectively. Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck). Microwave reactions were performed using the Biotage Initiator 2.0 single-mode cavity instrument from Biotage (Uppsala). Experiments were carried out in sealed microwave process vials utilizing the standard absorbance level (400 W maximum power).

Detailed synthetic procedures and characterization of all the compounds can be found in the Supplementary data.

2.2. Virus strains and cells

Chikungunya virus (CHIKV) Indian Ocean strain 899 (Genbank FJ959103.1) was generously provided by Prof. C. Drosten (University of Bonn, Germany). Venezuelan equine encephalitis virus (VEEV) vaccine strain TC83, CHIKV strain LR2006_OPY1 (Genbank DQ443544.2) and the clinical isolates Venturini (Italy 2008), Congo 95 (2011) and St Martin (2013) were used in EPV in Marseille and are freely disposable from the European Virus Archive (https://www.european-virus-archive.com/). Sindbis virus (SINV, strain HRsp, GenBank J02363.1) and the Semliki Forest virus (SFV, Vietnam strain, GenBank EU350586.1) belong to the collection of viruses at the Rega Institute of Medical Research, Belgium. All viruses were propagated in African green monkey kidney cells [Vero cells (ATCC CCL-81) or Vero E6 (ATCC CRL-1586)].

Vero cells were maintained in cell growth medium composed of minimum essential medium (MEM Rega-3, Gibco, Belgium) supplemented with 10% Foetal Bovine Serum (FBS, Integro, The Netherlands), 1% L-glutamine (Gibco), and 1% sodium bicarbonate (Gibco). The antiviral assays were performed in the same medium but supplemented with 2% (instead of 10%) FBS. The E6 sub-clone of Vero cells were maintained in Eagles MEM (Gibco) supplemented with antibiotics, 1% glutamine, 1% non essential amino-acids