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Discovery of novel multi-target indole-based derivatives as potent and selective inhibitors of chikungunya virus replication

Maria Scuotto [a,f], Rana Abdelnabi [d,f], Selene Collarile [a,f], Chiara Schiraldi [a], Leen Delang [d], Antonio Massa [c], Salvatore Ferla [e], Andrea Brancale [e], Pieter Leyssen [d], Johan Neyts [d], Rosanna Filosa [a,b,*]

[a] Department of Experimental Medicine, University of Naples, Via Costantinopoli, 16, 80138 Naples, Italy
[b] Consorzio Sannio Tech, Via Appia, SNC, 82030 Apollosa (BN), Italy
[c] KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, B-3000 Leuven, Belgium
[d] Department of Chemistry, University of Salerno, Via Ponte don Melillo, 84084 Fisciano, SA, Italy
[e] School of Pharmacy and Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, Cardiff CF10 3NB, UK

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ABSTRACT

We recently identified indole derivatives (**IIIe** and **IIIf**) with anti-chikungunya virus (CHIKV) activities at lower micro molar concentrations and a selective index of inhibition higher than the lead compound Arbidol. Here we highlight new structural information for the optimization of the previously identified lead compounds that contain the indole chemical core. Based on the structural data, a series of indole derivatives was synthesized and tested for their antiviral activity against chikungunya virus in Vero cell culture by a CPE reduction assay.

Systematic optimization of the lead compounds resulted in *tert*-butyl-5-hydroxy-1-methyl-2-(2-trifluoromethysulfynyl)methyl)-indole-3-carboxylate derivative **IIc** with a 10-fold improved anti-CHIKV inhibitory activity (EC_{50} = 6.5 ± 1 μM) as compared to Arbidol demonstrating a potent, selective and specific inhibition of CHIKV replication with only a moderate cell protective effect against other related alphaviruses. The reported computational insights, together with the accessible synthetic procedure, pave the road towards the design of novel synthetic derivatives with enhanced anti-viral activities.

1. Introduction

Chikungunya virus (CHIKV) is a re-emerging arbovirus that belongs to the genus alphavirus of the *Togaviridae* family. An infection with this virus results in chikungunya fever (CHIKF), with symptoms starting generally 4–7 days after the mosquito bite. The infection is occurring in two phases, the first being acute, characterized by fever and joint pains, while the second stage is persistent (chronic), causing disabling polyarthritis.[1] Although during the mid-twentieth century epidemiological emergency of CHIKV was limited to Africa, the virus spread to India and South-East Asia starting from 2004, causing large scale epidemics. After that, CHIKV transmission was also reported for the first time in Europe. In December 2013, the first local transmission was observed in the Americas, resulting in over 1,5 million infected patients. To date, despite its serious nature, no drugs have been approved for the treatment of CHIKV, but promising in vitro results have been obtained with Arbidol (ARB), a drug that already has been licensed for the treatment of influenza A and B virus infections in Russia and China.[2]

So far, Arbidol has shown a wide range of activity against many RNA, DNA, enveloped and non enveloped viruses[3,4] and recently also against CHIKV replication in immortalized Vero cells or primary human fibroblasts (MRC-5 lung cells) (EC50 <10 μg/mL).[4] The exact mechanism of the anti-CHIKV activity of Arbidol is not entirely understood. A previously reported study demonstrated its involvement in blocking the early stages of the viral life cycle.[5] Moreover, a single mutation from glycine to arginine in the E2 protein (G407R) generated an Arbidol resistant-CHIKV mutant giving an important insight of the mechanism of action. Gly407 is localised in the "wings" insertion of domain A, a region that could be involved in the interaction with different receptors.[5]

In our ongoing projects to elucidate the structural requirements of natural compounds or licensed drugs for their pharmacological activity,[6–19] we selected ARB for its broad antiviral activity, aiming

* Corresponding author at: Department of Experimental Medicine, Second University of Naples, Via Costantinopoli, 16, 80138 Naples, Italy.
E-mail address: rosanna.filosa@unina2.it (R. Filosa).
[f] These authors contribute equally.

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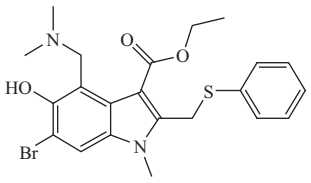
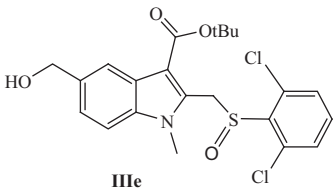


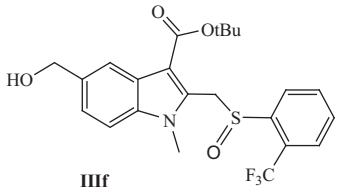
Arbidol



IIIe

EC_{50}: 30±4 CC_{50}: 397±24

SI: 13.2



IIIf

EC_{50}: 32±1.1 CC_{50}: > 468

SI: 14.6

Chart 1.

to improve the ARB therapeutic index, or to identify novel lead compounds active against HA viruses. Starting from Arbidol we identified several indole derivatives that inhibit CHIKV replication in phenotypic virus cell-based assays.[20] We demonstrated that the combined effect of an increased steric hindrance on the thiophenol ring and the replacement of an ethyl group in position 2 with a *tert*-butyl were necessary to improve the in vitro antiviral potency of this class of compounds. Moreover, oxidation of the sulfur atom in the linker led to a reduced cellular toxicity while preserving or increasing the antiviral activity. In particular, two new compounds **IIIe** and **IIIf** (Chart 1) had an antiviral activity higher than the other derivatives (EC_{50} 30–32 μM) with a selective index of inhibition 13.2 and 14.6 respectively, which is higher than Arbidol.

Here we describe the optimization of the previously identified indole derivatives based on novel structural information using the crystal structure of the CHIKV glycoprotein complex and the characterization of the mechanism of action of the anti-CHIKV activity of this series of compounds.

2. Results and discussion

2.1. Docking studies

In order to elucidate the potential binding pocket of Arbidol in the E2 viral protein and to suggest a hypothetical binding mode for the new indole derivatives, different molecular Modeling studies were performed. The E2 unit of the crystal structure of the mature E3-E2-E1 glycoprotein complex (PDB ID: 3N42) was used in the simulations (Gly407 corresponds to Gly82 in the crystal structure).[21] Using the site finder tool in MOE 2015.4, two potential binding sites in proximity of Gly82 were identified (site 1 and site 2; Fig. 1).

Docking studies of Arbidol on site 1, the closest site to Gly82, gave inconsistent results, indicating that this small hydrophilic and shallow pocket is probably not the binding site for Arbidol (Fig. 2b). Site 2 consists of a more hydrophobic and well-defined pocket formed by Trp64, Arg80, Met97, Thr96 and Thr160 (Fig. 2a). Gly82 is not directly involved in site 2 formation but it should be noted that this residue is located in a very flexible loop and it can be speculated that a substitution to arginine, a significantly bigger residue, could change the loop conformation affecting the overall architecture of the proposed site 2. Moreover, Arbidol has been reported to interact with tryptophan-rich molecules.[22]

Taking this into account, site 2 was considered the most relevant for our docking simulations. The results showed that Arbidol occupies the binding site inserting the ethyl ester group and the thiophenol deep in the pocket, whereas the dimethyl amino moiety and the hydroxyl group are more solvent exposed. Moreover, contacts between the ethyl ester and Trp64 and Arg80 are observed.

A different binding mode seems to be prevalent for the previously reported derivatives **IIIe** and **IIIf**. In this case, the presence of the bulkier *t*-butyl ester and the differently substituted thiophenols do not allow the same orientation as Arbidol. The indole ring of these derivatives sits deeper in the active site, in proximity of Trp64, while the differently substituted thiophenol occupies the more solvent exposed portion of the site, the same occupied by the indole ring of Arbidol. Finally, as shown in Fig. 3a and b, the *t*-butyl group of compound **IIIe** and **IIIf** occupies a small hydrophobic sub-pocket, formed by Thr160 and Thr96, possibly increasing the binding affinity. This correlates well with the increased biological activity often observed with the analogs that contain this ester.

Based on the above results, the design and synthesis of indole based molecules as new potential entry inhibitors was continued. In particular, from a structural point of view, the synthesized molecules can be assigned to three main groups:

(I) Indole *t*-butylester derivatives with the replacement of bromine in position 5 and phenol group in position 4 as the lead Arbidol,
(II) The most interesting compounds of series **I** were converted again into corresponding sulfoxides to confirm oxidation of sulfide to lead a reduction in cellular toxicity while preserving or increasing antiviral properties.
(III) Selected derivatives from the two previous series were analyzed regarding the simultaneously introduction of amine group and thiol oxidation.

We decided to use the same structural decorations in each subset of compounds in order to enable the identification of structural elements required to increase the antiviral effects and to confirm that the replacement of a hydroxyl group in position 5 is necessary to improve the in vitro antiviral potency of this class of compounds.