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Structure–activity relationship study of arbidol derivatives as inhibitors of chikungunya virus replication

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ABSTRACT

Chikungunya virus (CHIKV), a mosquito-borne arthrogenic *Alphavirus*, causes an acute febrile illness in humans, that is, accompanied by severe joint pains. In many cases, the infection leads to persistent arthralgia, which may last for weeks to several years. The re-emergence of this infection in the early 2000s was exemplified by numerous outbreaks in the eastern hemisphere. Since then, the virus is rapidly spreading. Currently, no drugs have been approved or are in development for the treatment of CHIKV, which makes this viral infection particularly interesting for academic medicinal chemistry efforts.

Several molecules have already been identified that inhibit CHIKV replication in phenotypic virus-cell-based assays. One of these is arbidol, a molecule that already has been licensed for the treatment of influenza A and B virus infections. For structural optimization, a dedicated libraries of 43 indole-based derivatives were evaluated leading to more potent analogues (**IIIe** and **IIIf**) with anti-chikungunya virus (CHIKV) activities higher than those of the other derivatives, including the lead compound, and with a selective index of inhibition 13.2 and 14.6, respectively, higher than that of ARB (4.6).

1. Introduction

Chikungunya virus (CHIKV) is an emerging mosquito-borne *Alphavirus* that causes debilitating rheumatic disease in humans. Acute illness includes fever, skin rash and incapacitating arthralgia which may last for weeks to months.[1] Since its first isolation from a febrile human in Tanzania in 1953, the virus has been re-isolated repeatedly from patients in numerous countries in Africa and South East Asia. In 2004, a CHIKV outbreak in the Indian Ocean region affected millions of people and infected travelers introduced CHIKV to new regions, including Europe and, more recently, Central America.[2] Although the virus in Africa is primarily maintained within a sylvatic cycle with wild mosquitoes preferentially feeding on primates,[3] CHIKV in Asia is mainly transmitted in a human-vector-human urban cycle by the *Aedes aegypti* mosquito.[4] In the Indian Ocean setting, *Aedes albopictus* appears to be the major vector, most likely with humans as a unique host.[5]

Current treatment of chikungunya virus infection is limited to alleviation of the symptoms of the disease. There is no effective licensed vaccine or specific antiviral drug available. The antimalarial drug chloroquine proved to be poorly active in vivo despite its potent antiviral effect on CHIKV replication in cell culture.[6] Similarly, it has been shown that the combination of interferon-alpha and ribavirin is effective against CHIKV in vitro but these compounds have not been evaluated for efficacy in animal models and/or clinical trials.[7] Arbidol (ARB, Fig. 1) is an antiviral drug that was originally licensed in Russia for the prophylaxis and treatment of infections with influenza A and B viruses. The compound has broad-spectrum antiviral activity against a number of enveloped and non-enveloped viruses, such as the hepatitis C virus. Recently, the in vitro antiviral effect of ARB on CHIKV replication was demonstrated in immortalized Vero cells or primary human fibroblasts (MRC-5 lung cells) (EC_{50} <10 μg/mL) by de Lamballerie and coworkers.[8]

In our ongoing project to elucidate the structural requirements of natural compounds or licensed drugs with anti-inflammatory and antiviral activity[9–16], we selected ARB for its broad antiviral activity, aiming to improve the ARB therapeutic index, or to identify novel lead compounds active against HA viruses. In particular

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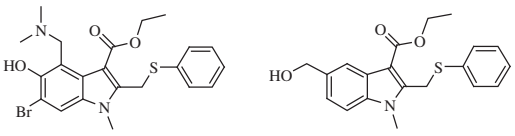


Arbidol A-15

Figure 1. Chemical structures of arbidol and **15**.

we identified arbidol derivatives with improved anti-influenza activity and with lower cytotoxicity, two series of compounds (Series **A** and **B**) were synthesized and evaluated for activity against a range of influenza viruses. Of all the compounds, ethyl 5-(hydroxymethyl)-1-methyl-2-((phenylthio)methyl)-1H-indole-3-carboxylate. (**A-15**) (Fig. 1) was found to be one of the most potent inhibitors, with a therapeutic index greater than arbidol for most viruses in the test panel. It exhibited a much greater affinity and preference for binding group 2 than group 1 HAs, and exerted a greater stabilizing effect, in contrast to arbidol.[17]

In this work, we evaluated the ability of these and other structurally related compounds (Series **C**)[18] to inhibit CHIKV replication in Vero cells (chemical structures and biological data are reported in Supplementary material). In the preliminary screening, compound **A-15** has shown to possess a weak antiviral effect on CHIKV replication (EC_{50} >157 μM). Compounds of series **B**, which bear an amino substituent in position 5, are completely inactive against CHIKV except for compound **B-28c**, which has an EC_{50} of 3.6 μM but a very low selectivity index (1.9), indicative of a significant adverse effect on the cells.

Regarding the compounds of Series **C**, in which amines in position 4 and bromine in position 6 have been eliminated and different substituents have been inserted on the thiophenol ring, all compounds are completely inactive.

Based on these results, new derivatives of arbidol were synthesized to investigate the role of the ethyl ester and substituents on the thiophenol ring in the context of CHIKV replication. In particular, small focused libraries (Series **I**–**II**) were prepared using straightforward synthesis pathways (Fig. 2). In the first series (**Ib**–**g**), several substituents with different properties were linked to the thiophenol ring. In the second series (**IIa**–**j**), replacement of the ethyl group in position 2 with a *tert*-butyl was also carried out to assess the combined effect of increased steric hindrance and lipophilicity.

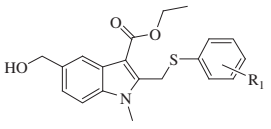
Moreover, taking into account the preliminary results, which showed significant cellular cytotoxicity for the compounds that belong to the *tert*-butyl series, derivatives with the most interesting EC_{50} values were converted into their corresponding sulfoxides (Series **IIIa**–**f** and **IVa**–**b**): Chai et al. demonstrated that the oxidation of the sulfide led to a reduced cellular toxicity while preserving or increasing the antiviral properties.[19] In the fifth series, the *tert*-butyl ester was hydrolized to verify if carboxylic acids (**Vd** and**Vh**) retain activity.

2. Results and discussion

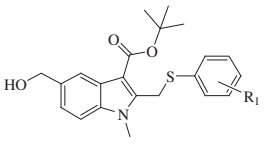
2.1. Chemistry

Alcohol derivatives **Ib**–**g** and **IIa**–**j** were synthesized according to Scheme 1. The synthesis of 5-hydroxymethyl-indole derivatives started from commercially available 4-ethyl aminobenzoate **1**, which was easily iodinated in position 2,[20] giving compound **2** in high yields. It was reacted with two different β-ketoesters (ethylacetoacetate or *tert*-butyl acetoacetate) in a copper-catalyzed Ullmann-type coupling reaction[21] giving the key intermediates **3** and **4**. Subsequent *N*-alkylation with iodomethane using a modified Kikugawa's procedure[22] afforded compounds **5** and **6** in quantitative yields. Treatment with 1 equiv of NBS allowed to obtain selective allylic brominated[23] compounds (**7** and **8**) and subsequent treatment with several substituted thiophenols in THF provided esters **9b**–**g** and **10a**–**j**. Reduction with DIBAL-H 1 M in CH_2Cl_2 at 0 °C allowed to recover alcohols **Ib**–**g** and **IIa**–**j**. The synthesis of sulfoxides **IIIa**–**f** and **IVa**–**b** is described in Scheme 2. Alcohol derivatives **A-15**, **Ig** and **IIa**, **IIc**–**e**, **IIg** and **IIh** were oxidized to sulfoxides using 77% meta chloroperbenzoic acid in CH_2Cl_2 at room temperature in very short time (5 min). Treatment of *tert*-butyl-5-ethyl-1,2-dimethyl-1*H*-indol-3,5-dicarboxylate (**6**) with 2.25 equiv of NBS in presence of dibenzoyl peroxide allowed to perform bromination of the allylic methyl group and simultaneous hydrolysis of

Series **I**



Series **II**



Cpd	R_1	Cpd	R_1
		IIa	H
Ib	4-OCH_3	**IIb**	4-OCH_3
Ic	4-F	**IIc**	4-F
Id	4-CF_3	**IId**	4-CF_3
Ie	4-OCF_3	**IIe**	4-OCF_3
If	2,4-F	**IIf**	2,4-F
Ig	2,6-Cl	**IIg**	2,6-Cl
		IIh	4-Cl
		IIi	2,4,6-CH_3
		IIj	2-CF_3

Figure 2. Chemical structures of new arbidol derivatives: Series I and Series II.