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Thiazolidone derivatives as inhibitors of chikungunya virus

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ABSTRACT

A series of arylalkylidene derivatives of 1,3-thiazolidin-4-one (**1**–**20**) were synthesized and tested for their antiviral activity against chikungunya virus (LR2006_OPY1) in Vero cell culture by CPE reduction assay. Five compounds (**7**–**9**, **16** and **19**) were identified to have anti-ChikV activity at lower micro molar concentration. The compounds **7**, **8, 9**, **16** and **19** inhibited the virus at 0.42, 4.2, 3.6, 40.1 and 6.8 μM concentrations respectively. Molecular docking simulation has been carried out using the available X-ray crystal structure of the ChikV nsp2 protease, in order to elucidate the possible mechanism of action. Interaction of ligands with ChikV nsp2 protease (PDB Code: 3TRK) suggested the possible mechanism of protease inhibition to act as potent anti-ChikV agents.

1. Introduction

Chikungunya, an emerging arthropod-borne viral infection caused by the chikungunya virus (ChikV, an arbovirus) was first reported from Tanzania during 1952 [1]. A major outbreak has been reported more than 50 years later, during 2005–2007 in Africa and Asia [2,3] that was followed by limited outbreaks in Europe [4] and the US [5].The emergence of a new clinical form of the virus [6] with vector adaptation (*Aedes albopictus*) [7] explains its geographical spread to developed nations. Threat due to this emerging virus is likely to be high in future if no means to prevent/treat the infection is developed and made available. To date, there is no effective vaccine or chemotherapeutic agent available.

Interferons [8] and their combination with Ribavirin [9] and Mercaptopurine [10] were reported to have antiviral activity against ChikV. Arbidol, an antiviral licensed for the treatment of influenza, was found to inhibit ChikV replication [11]. Extracts of a few plant materials have also been reported to exert anti-ChikV activity [12–18]. The current investigation presents the anti-ChikV activity of benzylidene rhodanine derivatives since rhodanine has been identified as a privileged scaffold [19] and reported with antiviral activity against HCV [20,21] and HIV [22]. Molecular docking simulation has been carried out with the recently deposited X-ray crystal structure of Chikv nsp2 protease (PDB Code: 3TRK) in order to understand the mechanism of action of the active molecules.

2. Results and discussion

2.1. Chemistry

A series of twelve arylalkylidene derivatives of 1,3-thiazolidin-4-one (**1**–**20**) were synthesized following the reaction outlined in Schemes 1 and 2 [23]. Knoevenagel condensation of 2-sulfanylidene-1,3-thiazolidin-4-one (rhodanine) with aromatic/heteroaromatic aldehydes in the presence of acetic acid and ethanol provided compounds **1**–**12** (Scheme 1). Similarly, condensation of 2-amino-4,5-dihydro-1,3-thiazole-4-one (pseudothiohydantoin) with aromatic/heteroaromatic aldehydes in the presence of ammonium acetate and glacial acetic acid provided compounds **13**–**20** (Scheme 2). The resultant precipitates were recrystallized with ethanol to obtain pure final product. All the final compounds were found to have melting points closely matching

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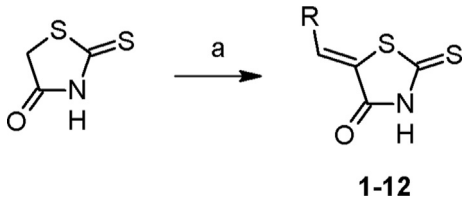
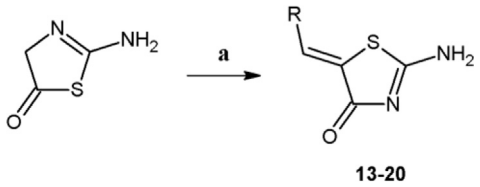


Scheme 1. Reagents and conditions: (a) R–CHO, EtOH, AcOH, reflux, 24 h.



Scheme 2. Reagents and conditions: (a) R–CHO, EtOH, AcOH, NH_4Ac, reflux, 24 h.

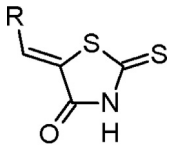
with the available literature and further their structures were confirmed by their ^1H NMR, ^{13}CNMR and MS data.

All the compounds were screened for their anti-ChikV and cytotoxic activity following the procedures discussed below.

2.2. Anti-Chikv & cell viability assay

Anti-ChikV assay has been carried out with ChikV strain LR2006_OPY1 in Vero cell culture by CPE reduction assay. All the twenty (**1**–**20**) compounds have been evaluated for their antiviral activity and five compounds (**7**–**9**, **16** and **19**) were found to have antiviral activity (Tables 1 and 2, Graphs in Supplementary material). Three compounds (**7**–**9**), aralkylidene derivatives of 2-sulfanylidene-1,3-thiazolidin-4-one (**1**–**12**) were found to be active. Compound **7** with *ortho*-methyl substitution (IC_{50} = 0.1 μg/mL, Table 1) was found most potent amongst the three and was followed by compound **8** with *para*-methyl substitution (IC_{50} = 1.0 μg/mL, Table 1). Methyl substitution was favorable when it was at *ortho*-position (**7**) and was found to be ten times more potent than its *para*-counterpart (**8**). Compound **9** (IC_{50} = 1.0 μg/mL, Table 1) with 2-napthyl ring inhibited virus replication at a concentration similar to that of compound **7**. This clearly indicated that aralkylidene portion should be non-polar in nature to exert activity. This is further supported by the fact that compounds **1**–**6** with polar substitutions at *para*-position and compounds **10**–**12** with heteroaryl rings were found inactive at highest concentration studied (100 μg/mL). Two compounds (**16** & **19**, Table 2) from aralkylidene derivatives of 2-amino-4,5-dihydro-1,3-thiazole-4-one (**13**–**20**, Table 2) were found to be active. Compound **16** with *ortho*-nitro substitution and compound **19** with *meta*-methyl substitution were found active at concentrations IC_{50} = 10.0 μg/mL and IC_{50} = 1.5 μg/mL, respectively (Table 2). Compound **6** and **16** differ in substitution at 2nd position of thiazolidine ring. A compound featuring sulfanylidene (**6**) was found inactive while the other with amino group (**16**) was found to exhibit activity. Similarly, compounds **7** and **8** differ from compound **18** and **20** at 2^{nd}-position, but here the compounds having sulfanylidene group (**7** & **8**) were found to be active and compounds with amino group (**18** & **20**) were found inactive. Foregoing observation clearly indicates the influence of endo/exo nature of double bond involving 2nd position of thiazolidine and substitution in the phenyl ring of aralkylidene portions on the activity of the molecules. In the next Section (2.3) interaction at molecular level will be discussed having simulations carried out with ChikV-nsp2 protease as a possible target.

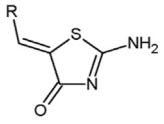
Table 1
Antiviral activity of Compounds **1**–**12** against ChikV.



Code	R	IC_{50} (μM)	CC_{50} (μM)
1	4-OH-C_6H_4	ND	>100
2	4-OCH_3-C_6H_4	ND	>100
3	4-Cl-C_6H_4	ND	>100
4	4-$N(CH_3)_2$–C_6H_4	ND	>100
5	4-CN-C_6H_4	ND	>100
6	2-NO_2-C_6H_4	ND	>100
7	2-CH_3-C_6H_4	0.42 (0.1 μg/mL)	>100
8	4-CH_3-C_6H_4	4.2 (1.0 μg/mL)	>100
9	$C_{10}H_7$ (napth-2-yl)	3.6 (1.0 μg/mL)	>100
10	C_4H_3S (thiophen-2-yl)	ND	>100
11	C_5H_4N (pyridine-2-yl)	ND	>100
12	C_5H_4N (pyridine-3-yl)	ND	>100

ND-Not Determined (Not showing any activity at the maximum concentration studied ie. 100 μM).

Table 2
Antiviral activity of Compounds **13**–**20**against ChickV.



Code	R	IC_{50} (μM)	CC_{50} (μM)
13	3-OH-C_6H_4	ND	>100
14	4-OH-C_6H_4	ND	>100
15	2,4-diOH-C_6H_3	ND	>100
16	2-NO_2-C_6H_4	40.1 (10.0 μg/mL)	>100
17	3-NO_2-C_6H_4	ND	>100
18	2-CH_3-C_6H_4	ND	>100
19	3-CH_3-C_6H_4	6.8 (1.5 μg/mL)	>100
20	4-CH_3-C_6H_4	ND	>100

ND-not determined (Not showing any activity at the maximum concentration studied ie. 100 μg/mL).

Active molecules did not show any cytotoxic effect at their active concentrations. Microscopic observation revealed no changes in the host cell morphology, which clearly indicates the antiviral property of the compounds analyzed (Potential cytotoxic/cytostatic effects of the compound were evaluated in uninfected cells by observing microscopically for any minor signs of virus-induced CPE or alterations to the cells caused by the compound).

2.3. Molecular docking simulation

In an attempt to understand the possible mechanism of action of the active compounds, molecular docking simulation has been carried out with the X-ray crystal structure of Chikv nsp2 protease (PDB Code: 3TRK). For compound **7**, the aralkylidene portion of the molecule shows strong hydrophobic interaction with three amino-acid residues (TYR1047, TYR1049 and TRP1084) in S3 pocket (formed by TYR1047, TYR1049, TRP1084, MET1238, MET1242) while the thiazolidinone portion shows strong hydrophobic