Microwave-assisted synthesis and anti-YFV activity of 2,3-diaryl-1,3-thiazolidin-4-ones

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ABSTRACT

Purpose: The purpose of this study was to prepare several 1,3-thaizolidin-4-ones bearing variously substituted diaryl ring at C-2 and N-3 positions and evaluate them for their anti-YFV activity. **Methods**: Several 1,3-thaizolidin-4-ones were prepared by reacting substituted benzaldehyde with equimolar amount of an appropriate substituted aromatic amine in the presence of an excess of mercaptoacetic acid in toluene utilizing microwave irradiation. The synthesized compounds were also evaluated for their inhibitory effects on the replication of YFV in green monkey kidney (Vero) cells (ATCC CCL81), by means of a cytopathic effect reduction assay. **Results**: The compound DS1 emerged as the most potent anti-YFV agent with EC_{50} of 6.9 μM and CC_{50} more than 100 μM making it more potent than ribavirin. **Conclusion**: 2,3-diaryl-1,3-thiazolidin-4-ones possess anti-YFV potency.

INTRODUCTION

Yellow fever is a serious viral infection, transmitted by mosquitoes in tropical regions. Yellow fever virus [YFV] belongs to the Flaviviridae family [1], The virus is introduced into the bloodstream via the saliva of the mosquito Aedes egyptii [2] The virus is permanently prevalent, with a more or less constant number of sufferers (i.e. it is endemic) in several tropical regions of Africa and on the continent of America. In addition, there is an increasing number of epidemics, in which a large number of people suddenly develop yellow fever. Every year about 200,000 cases of yellow fever are recorded, and 30,000 of those infected die, but the figures are underestimated because of poor record keeping. In total, yellow fever occurs in 33 countries and 468 million people are at risk of catching the disease [3]. Once a mosquito has passed the yellow fever virus to a human, the chance of developing clinical disease is about 5-20%. Symptoms of the disease [3] include high fever, anemia, as well as liver inflammation, hepatitis and jaundice. The kidneys are also affected and bleeding from the mouth, nose and stomach may occur, which leads to blood in vomit and faeces. Because of this degeneration of the internal organs (specifically the kidneys, liver, and heart), it results in what is considered the classic triad of yellow fever symptoms: jaundice, black vomit, and the dumping of protein into the urine. Jaundice causes the whites of the patient's eyes and the patient's skin to take on a distinctive yellow color. This is due to liver damage, and the accumulation of bilirubin, which is normally processed by a healthy liver. There are no current anti-viral treatments available to combat the yellow fever virus. The only treatment of yellow fever involves attempts to relieve its symptoms. Fevers and pain should be relieved with acetaminophen not aspirin or ibuprofen, both of which could increase the already-present risk of bleeding. The drugs which are useful for YFV infection include Ribavirin [4] and α-Interferon [4]. Other compounds which show anti-YFV activity include 3-deazaneplanocin-A [5], sulfated galactomannans [6] phosphorothioate oligodeoxyribonucleotides [7] and selenazofurin [8]. From a random screening program of compounds having anti-HIV activity [9-12], we identified a series of 1,3-thaizolidin-4-ones bearing variously substituted diaryl ring at C-2 and N-3 positions as inhibitors of YFV. In this communication, we describe the efficient synthesis and preliminary anti-YFV activity of novel compounds having the thiazolidinone skeleton.

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EXPERIMENTAL

*General Procedure for the synthesis of 2,3-diaryl-1,3-thiazolidin-4-ones (**DS1-DS15**)*

To a stirred solution of aromatic amine (0.01 mole) in dry toluene (50 mL), 2-mercaptoacetic acid (0.02 mole) and the appropriate aromatic aldehyde (0.01 mole) were added and irradiated in an unmodified domestic microwave oven at power setting of 80% with 30 seconds/cycle. The number of cycles in turn depended on the degree of completion of the reaction, which was checked by thin layer chromatography (TLC). The reaction timing varied from 6 to 8 minutes. The solution obtained after the completion of the reaction was kept at 0°C for 30 minutes. During this time the excess of unreacted mercaptoacetic acid was frozen out at the bottom and the clear solution was decanted. After the removal of solvent under reduced pressure, the oily residue was treated with a mixture of ethanol and diethyl ether to yield solid title compounds in the yields ranging from 64-82%. Physical and spectral data of representative compounds are as follows.

***DS1**: 2-(4'-Chlorophenyl)-3-(4"-fluorophenyl)-1,3-thiazolidin-4-one*
M.P.: 104°C; Yield: 68%; ^{1}H-NMR, δ ppm: 3.87 (d, 1H, 5-H_A), 4.09 (dd, 1H, 5-H_B), 7.06-7.35 (m, 9H, ArH and H-2). Anal. ($C_{15}H_{11}NOSClF$) C, H, N.

***DS6**: 3-(2,6-Dimethylphenyl)-2-(4"-nitrophenyl)-1,3-thiazolidin-4-one*
M.P.: 95°C; Yield:79%; ^{1}H-NMR, δ ppm: 1.1 (s, 6H, CH3), 3.86 (d, 1H, 5-H_A), 4.11 (dd, 1H, 5-H_B), 7.10-7.36 (m, 8H, ArH and H-2). Anal. ($C_{17}H_{16}N_2O_3S$) C, H, N.

***DS8**: 2-(4'-Chlorophenyl)-3-(2"-chloro-3"-methylphenyl)-1,3-thiazolidin-4-one*
M.P.: 121°C; Yield: 70%; ^{1}H-NMR, δ ppm: 2.1 (s, 3H, CH_3), 3.86 (d, 1H, 5-H_A), 4.18 (dd, 1H, 5-H_B), 7.06-7.46 (m, 8H, ArH and H-2). Anal. ($C_{16}H_{13}NOSCl_2$) C, H, N.

***DS13**: 2-(2'-Chlorophenyl)-3-pyridin-2-yl-1,3-thiazolidin-4-one*
M.P.: 100°C; Yield: 64%; ^{1}H-NMR, δ ppm: 3.84 (d, 1H, 5-H_A), 4.22 (dd, 1H, 5-H_B), 6.86-8.20 (m, 9H, ArH and H-2). Anal. ($C_{14}H_{11}N_2OSCl$) C, H, N.

***DS15**: 2-(4'Chlorophenyl)-3-(4"-methylpyridin-2-yl)-1,3-thiazolidin-4-one*
M.P.: 184°C; Yield: 65%; ^{1}H-NMR, δ ppm: 2.25 (s, 3H, CH_3), 3.92 (d, 1H, 5-H_A), 4.13 (dd, 1H, 5-H_B), 7.05-8.25 (m, 8H, ArH and H-2). Anal. ($C_{15}H_{13}N_2OSCl$) C, H, N.

Anti-YFV evaluation

Viruses and cells

Green monkey kidney (Vero) cells (ATCC CCL81) were grown in minimum essential medium (MEM, Gibco, Paisley, Scotland) supplemented with 10% inactivated calf serum (FCS, Gibco), 1% L-glutamine and 0.3% bicarbonate. The vaccine strain 17D (Stamavil®, Pasteur Merieux) was used to infect 75 mL bottles of Vero cells. After a 5-day incubation period at 37°C, extensive cytopathic effect was observed; the cultures were freeze-thawed, cell debris removed by centrifugation and the supernatant aliquoted and stored at -70°C.

Inhibition of virus induced cytopathogenicity

Serial dilutions of the test compounds were added to confluent Vero cell cultures in microtitre trays after which the cells were infected with 10$CCID_{50}$ (Cell culture infective dose 50%) of virus. Cultures were further incubated at 37°C. Viral cytopathogenicity was recorded 7-8 days post infection. Cultures were fixed with 70% ethanol, stained with Giemsa solution (50-fold dilution; 2h staining), washed and air-dried. The antiviral activity of the compound was expressed as the effective concentration required to inhibit the viral cytopathic effect by 50% (EC_{50}).

Cytotoxicity

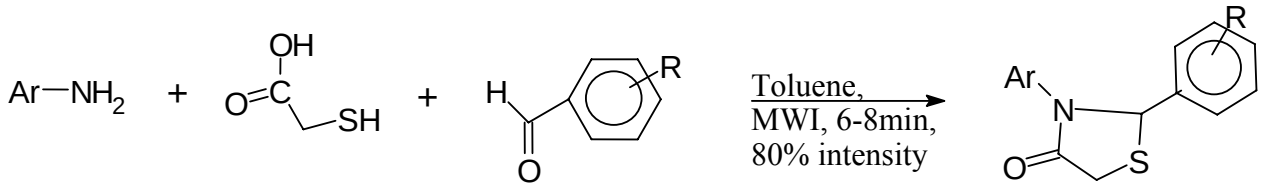
Inhibition of uninfected host cell growth was assessed as follows: the cells were seeded at a rate of 3 x 10^3 cells per well in a volume of 0.1 mL into 96-well microtitre plates and allowed to proliferate for 24h in MEM containing 20% FCS, 1% L-glutamine and 0.3% sodium bicarbonate. Twenty-four hours later, 0.1 mL MEM (with 2% FCS, 1% L-glutamine and 0.3% sodium bicarbonate) containing different concentrations of the test compounds were added to each well. After 3 days of incubation at 37°C in 5% carbon dioxide, the cell number was determined with Coulter counter. The minimum cytotoxic

concentration was expressed as CC_{50}, the concentration required to reduce cell growth by 50%.

RESULTS AND DISCUSSION

Chemistry

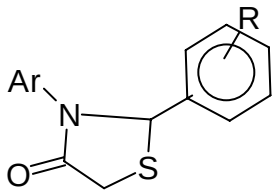
The synthesis of the 2,3-diaryl-1,3-thiazolidin-4-ones (**DS1-15**) was accomplished by reacting substituted benzaldehyde with equimolar amount of an appropriate substituted aromatic amine in the presence of an excess of mercaptoacetic acid in toluene utilizing microwave irradiation (Fig. 1). Unlike the conventional methods [9-12] (reaction time 48h and yields of 30-70%), microwave-assisted reactions were very facile (6-8 min.) and provided very good yields (64-82%). The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (^{1}H-NMR) of all the synthesized compounds were in full agreement with the proposed structures. The ^{1}H-NMR spectrum revealed that the 3.86-3.88 for 5-H_A (d, 1H), 4.09-4.11 for 5-H_B (dd, 1H) and 7.06-7.35 for Ar-H and H-2.



DS1-15

Figure 1: Synthesis of 2, 3-diaryl-1, 3-thiazolidin-4-ones

Table 1. Anti-YFV activities of 2, 3-diaryl-1, 3-thiazolidin-4-ones.



Compound	Ar	R	$EC_{50,}$ (μM)	$CC_{50,}$ (μM)
DS1	4-F-C_6H_4-	4-Cl	6.83	>100
DS2	4-F-C_6H_4-	2-Cl	7.9	100
DS3	4-F-C_6H_4-	3-NO_2	15	100
DS4	4-F-C_6H_4-	4-N$(CH_3)_2$	>100	100
DS5	4-F-C_6H_4-	2-OH	>100	100
DS6	2,6-$(CH_3)_2$-C_6H_3-	4-NO_2	76	100
DS7	2,6-$(CH_3)_2$-C_6H_3-	4-OH	76	100
DS8	3-Cl, 2-CH_3-C_6H_3-	4-Cl	>100	100
DS9	3-Cl, 2-CH_3-C_6H_3-	2-Cl	>100	100
DS10	3-Cl, 2-CH_3-C_6H_3-	4-N- $(CH_3)_2$	>100	100
DS11	3-Cl, 2-CH_3-C_6H_3-	2-OH	>100	100
DS12	2,6-$(Cl)_2$-C_6H_3-	4-NO_2	>100	100
DS13	2-C_5H_4N-	2-Cl	>100	100
DS14	2-C_5H_4N-	4-Cl	15	100
DS15	3-CH_3-2-C_5H_4N-	4-Cl	>100	>100

Anti-YFV activity

All these compounds were also evaluated for their inhibitory effects on the replication of YFV in green monkey kidney (Vero) cells (ATCC CCL81), by means of a cytopathic effect reduction assay [12]. The antiviral activity of the compound was expressed as the effective concentration required to inhibit the viral cytopathic effect by 50% (EC_{50}). The minimum cytotoxic concentration was expressed as CC_{50}, the concentration required to reduce cell growth by 50%. EC_{50} and CC_{50} values are presented in Table 1. Six compounds (**DS1-3, DS6-7** and **DS14**) were found to prevent the YFV infection of the cells at concentrations that had no effect on cell growth. Four compounds (**DS1-3** and **DS14**) were found to be more effective than ribavirin (EC_{50} values of 28.0 μM). The compound **DS1** emerged as the most potent anti-YFV agent with EC_{50} of 6.9 μM and CC_{50} more than 100 μM.

In the present study we have discovered 2, 3-diaryl-1, 3-thiazolidin-4-ones as a new lead in the anti-YFV field. These results need to be refined in terms of active concentration and toxicity. Further studies to acquire more information about structure-activity relationships are in progress in our laboratory.

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