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Thiosemicarbazones and Phthalyl-Thiazoles compounds exert antiviral activity against yellow fever virus and Saint Louis encephalitis virus

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ABSTRACT

Arboviruses, ***arthropod-borneviruses***, are frequency associated to human outbreak and represent a serious health problem. The genus Flavivirus, such as Yellow Fever Virus (YFV) and Saint Louis Encephalitis Virus (SLEV), are important pathogens with high morbidity and mortality worldwide. In Brazil, YFV is maintained in sylvatic cycle, but many cases are notified annually, despite the efficiency of vaccine. SLEV causes an acute encephalitis and is widely distributed in the Americas. There is no specific antiviral drugs for these viruses, only supporting treatment that can alleviate symptoms and prevent complications. Here, we evaluated the potential anti-YFV and SLEV activity of a series of thiosemicarbazones and phthalyl-thiazoles. Plaque reduction assay, flow cytometry, immunofluorescence and cellular viability were used to test the compounds *in vitro*. Treated cells showed efficient inhibition of the viral replication at concentrations that presented minimal toxicity to cells. The assays showed that phthalyl-thiazole and phenoxymethyl-thiosemicarbazone reduced 60% of YFV replication and 75% of SLEV replication.

1. Introduction

Flaviviruses are a group of mainly zoonotic viruses transmitted by mosquitoes, and include important human pathogens [1] and can cause a broad spectrum of disease in humans including hemorrhagic fever febrile illness or encephalitis [2]. More important, these viruses have a significant impact on public health systems in different parts of the world [3,4].

Yellow fever was the first human disease to be attributed to a virus and first demonstrated to be transmitted by an arthropod. [5,6]. Despite the existence of highly effective vaccines for over 70 years, yellow fever remains a significant and re-emerging cause of morbidity and mortality in endemic and high-risk regions of South America and Africa [7–9].

SLEV is widely distributed in the Americas, from Canada to Argentina [10,11]. Since SLEV was discovered, St. Louis encephalitis outbreaks have been reported in the United States [12–14], in contrast to Central and South America where outbreak reports are scarce, but probably underestimated [15–21].

Specific treatment is not available for yellow fever or St. Louis encephalitis and intensive supportive therapy is the sole treatment to prevent complications. Therefore, the development of effective antiviral drugs to reduce the morbidity and mortality caused by flaviviruses infection is a priority [22].

Thiosemicarbazones have a place of prominence within medicinal chemistry due to their wide spectrum of biological activities. Thiosemicarbazones present antiviral [23,24], antitumor [25], antibacterial [26], antifungal [27] and antiprotozoal activities [28]. Terzioğlu and colleagues [29] evaluated the activity of thiosemicarbazones and thiazolidinones against YFV in Vero cells and observed that the most active compound was an substituted

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allyl thiosemicarbazone derivative. Furthermore, 5-methyl-4-thiazolidinone derivatives present decreased antiviral activity against YFV, suggesting the importance of the pharmacophoric group for the antiviral property. Virtual screening of the National Cancer Institute chemical database recently identified a small set of compounds that were predicted to bind flavivirus E protein at a detergent-binding pocket. Compounds that inhibited YFV replication share the common feature of a central thiazole ring [30], indicating that this pharmacophoric group also presents anti-flavivirus activities.

An appropriately chosen molecular scaffold can increase hit rates at the biological target of interest, in addition to producing leads with enhanced drug-like properties. With this in mind, we evaluated antiviral effects of molecules whose structures have a thiosemicarbazone or a phthalyl-thiazole nucleus against YFV and SLEV, and whether these pharmacophores influence viral protein expression.

2. Material and methods

2.1. Cells and viruses

Vero E6 cells were grown in Eagle's Minimum Essential Medium (MEM, GIBCO, USA) supplemented with 10% Fetal Bovine Serum (FBS) (Cultilab, Brazil), 100 U/ml penicillin and 100 μg/mL streptomycin (GIBCO, USA), and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. C6/36 cells were maintained in Leibovitz-15 medium (L-15) with 10% FBS at 28 °C. YFV vaccine strain 17DD (FIOCRUZ, Brazil) and SLEV strain BeH355964 (provided by Dr. Luis Tadeu Figueiredo, University of São Paulo, Brazil) [31] were propagated in C6/36 cells and titrated by plaque assay in Vero E6 cells. Viral titers were expressed as plaque forming units (PFU) per mL.

2.2. Compounds

Compounds 2-(3-chlorophenoxy) acetaldehydethiosemicarbazone (**1**), 2-(3,4-dichlorophen oxy) acetaldehydethiosemicarbazone (**2**) and 2-(2,3-dichlorophenoxy) acetaldehyde thiosemicarbazone (**3**) were prepared as described by Moreira et al. [32]. Compounds 2-(4-(4-fluorophenyl)thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (**4**) and 2-(4-(4-chlorophenyl) thiazol-2-yl)hydrazono)ethyl)isoindoline-1,3-dione (**5**) were prepared as described by Pessoa et al. [33]. All compounds (Fig. 1) were chemically characterized by nuclear magnetic resonance (NMR), infrared, mass spectra and by elemental analysis and presented purity of 95%. The cytotoxicity of the compounds was evaluated in lymphocytes, macrophages and splenocytes cells lines [34,35].

2.3. Plaque reduction assay

The assay was performed as described previously [36] with minor modifications. 8×10^5 cells Vero E6 cells were plated into

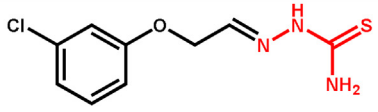
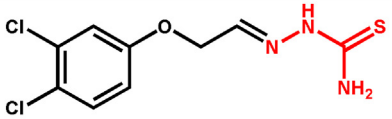
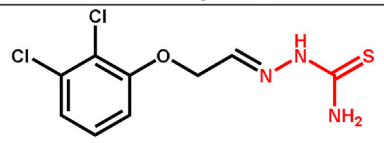
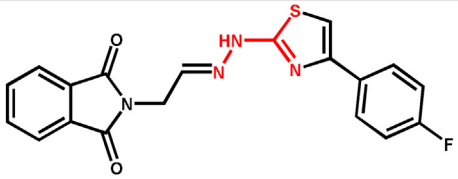
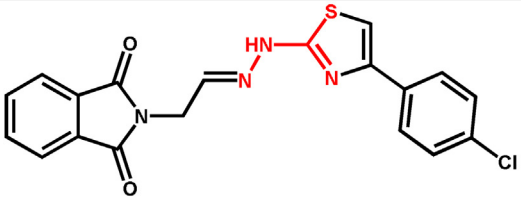
Analog		Compound
Phenoxy-thiosemicarbazone	 Molecular Weight: 243,7090	1
Phenoxy-thiosemicarbazone	 Molecular Weight: 278,1510	2
Phenoxy-thiosemicarbazone	 Molecular Weight: 278,1510	3
Phthalyl-thiazole	 Molecular Weight: 380,3974	4
Phthalyl-thiazole	 Molecular Weight: 396,8490	5

Fig. 1. Thiosemicarbazone and thiazole compounds evaluated for antiviral activity against flaviviruses.