Antiviral and anti inflammatory activity of synthetic steroidal analogues against the infection caused by Human Respiratory Syncytial Virus

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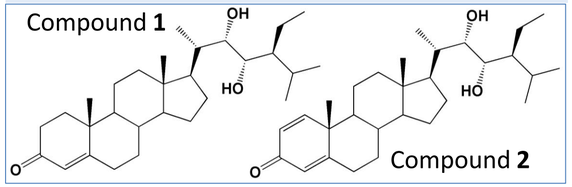
INTRODUCTION

Compounds (22S,23S)-22,23-dihydroxystigmast-4-en-3-one (**1**) and (22S,23S)-22,23-dihydroxystigmasta-1,4-dien-3-one (**2**) are two stigmastane analogs with antiviral activity against non-related viruses of clinical relevance, with different structures and replicative strategies. Our aim was to evaluate their potential inhibitory activity against Respiratory Syncytial Virus (RSV),), known to be the leading cause of lower respiratory tract disease and bronchiolitis in children worldwide.



AIMS

☼ To investigate the *in vitro* antiviral activity of Compounds **1** and **2** against RSV A2 y L19 strains in A549, Hep-2 and Vero cells.

☼ To study the *in vitro* immunomodulatory activity of the compounds in murine and human macrophages infected with RSV A2 and L19 strains.

☼ To investigate the antiviral and anti inflammatory activity of the compounds in an *in vivo* model of infection with RSV L19 strain.

RESULTS

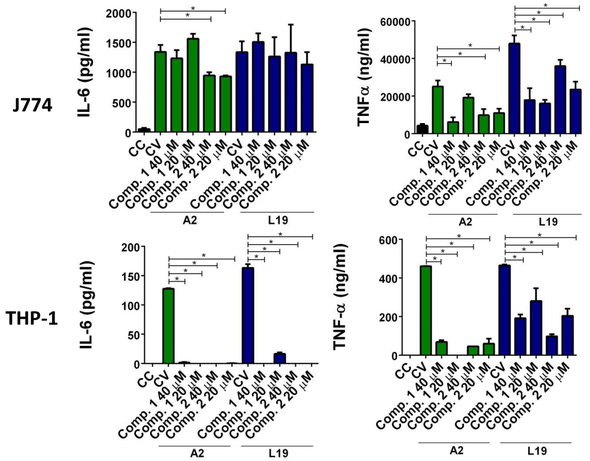
In vitro antiviral activity

	COMPOUND 1				COMPOUND 2			
	A2		L19		A2		L19	
	EC50 (µM)	SI	EC50 (µM)	SI	EC50 (µM)	SI	EC50 (µM)	SI
HEp-2	5.6	3.9	8.5	2.5	5.8	2.7	5.1	3
A549	3.5	16	6	8.8	1.8	> 116	6.1	> 32.5
Vero	14.8	6.7	12	8.2	5.4	10.4	5.2	10.8

EC_{50}: Effective Concentration 50%.
SI: Selectivity index.

Cytotoxicity assay: Cell monolayers were treated with different concentrations of the compounds and incubated for 24 h at 37 °C. Cell viability was determined by the MTT assay. The Cytotoxic Concentration 50% was defined as the concentration of compound that caused a 50% reduction in cell viability.
Antiviral activity assay: cell monolayers were infected with RSV A2 and L19 strains (moi = 1) and treated with different concentrations of the compounds or control media, in triplicate. After 24 h of incubation at 37 °C, infected cultures were frozen and thawed, and the samples were titrated in Vero cells by a plaque assay. The Effective Concentration 50% was defined as the concentration of compound that caused a 50% reduction in viral yields, with respect to untreated virus control.
Selectivity index = CC_{50}/EC_{50}.

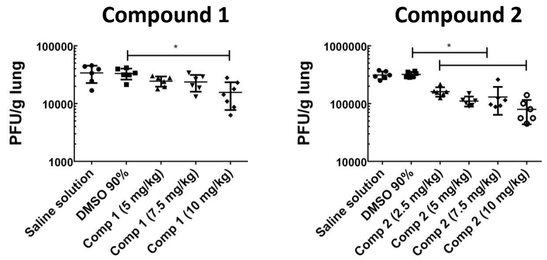
In vitro immunomodulatory activity



J774A.1 cells were infected with RSV A2 and L19 strains (moi = 1). After virus adsorption, cells were incubated at 37 °C in the presence or absence of the compounds for 24 h. Supernatants were harvested and IL-6 and TNF-α were determined by ELISA. *Significantly different from RSV infected cells (CV).

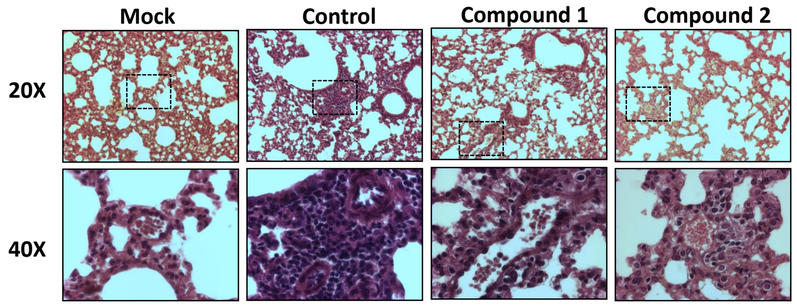
THP-1 cells were incubated with 50 ng/ml PMA for 72 h and then they were infected with RSV A2 and L19 strains (moi = 1), in triplicate. After virus adsorption, cells were incubated at 37 °C in the presence or absence of the compounds for 24 h. Supernatants were harvested and IL-6, TNF-α and IL-8 were determined by ELISA. *Significantly different from RSV infected cells (CV).

In vivo antiviral and anti inflammatory activity



Female Balb/c mice were infected with RSV L19 strain (5×10^6 PFU) by intranasal instillation, concomitant with different doses of the compounds dissolved in DMSO 90% by intraperitoneal (i.p.) injection on day 0. On days 1–4, all mice received further inoculations of the compounds i.p.

A group of animals were killed on day 4 post infection and the lungs were used for titration of infectious virus. Data show mean ± SD from n = 6 mice/condition. *Significantly different from RSV infected mice treated with vehicle (p-value<0.05).



A group of animals were killed on day 8 post infection and histopathology was assessed: lungs were collected and stained for H&E. Light micrographic images shown at 20X and 40X, representative of n=3/condition.

DISCUSSION

- Compounds **1** and **2** inhibited RSV A2 and L19 strains replication *in vitro*, in the three cell lines tested, with EC_{50} between 3 µM and 15 µM, with higher SI observed in A549.
- Both compounds significantly reduced cytokine production in murine and human macrophages infected with RSV A2 and L19 strains.
- A concentration of 10 mg/kg of both compounds reduced viral loads in the lungs of mice infected with RSV L19 strain. Compound **2** was also effective to reduce viral loads when it was administered at lower doses. Both compounds reduced lung inflammation and inflammatory cell infiltration.
- Both compounds are promissory as antivirals and anti inflammatory drugs to ameliorate the respiratory disease caused by RSV.