

# Candidate antivirals for treatment of respiratory syncytial virus and coronavirus infections

## Identification and elucidation of mode of antiviral activity

Akademisk avhandling

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av

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This thesis is based on the following studies

- I. **Lundin A**, Bergström T, Bendrioua L, Kann N, Adamiak B, Trybala E. Two novel fusion inhibitors of human respiratory syncytial virus. *Antiviral Res* 2010; 88(3):317-24.
- II. **Lundin A**, Bergström T, Andrighetti-Fröhner CR, Bendrioua L, Ferro V, Trybala E. Potent anti-respiratory syncytial virus activity of a cholestanol-sulfated tetrasaccharide conjugate. *Antiviral Res* 2012; 93(1):101-9.
- III. **Lundin A**, Bergström T, Trybala E. Screening and evaluation of anti-respiratory syncytial virus compounds in cultured cells. *Methods Mol Biol* 2013; 1030:345-63.
- IV. **Lundin A**, Dijkman R, Bergström T, Kann N, Adamiak B, Hannoun C, Kindler E, Jónsdóttir HR, Muth D, Kint J, Forlenza M, Müller MA, Drosten C, Thiel V, Trybala E. Targeting membrane-bound viral RNA synthesis reveals potent inhibition of diverse coronaviruses, including the Middle East respiratory syndrome virus. *Submitted*.

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UNIVERSITY OF GOTHENBURG

# Candidate antivirals for treatment of respiratory syncytial virus and coronavirus infections

## Identification and elucidation of mode of antiviral activity

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### Abstract:

Respiratory syncytial virus (RSV) and coronaviruses (CoVs) are frequent causes of respiratory disease in humans. RSV can cause severe bronchiolitis and pneumonia in infants, especially in those born prematurely or with underlying cardiopulmonary chronic dysfunction. CoV respiratory illnesses can vary in severity ranging from common cold-like symptoms to severe respiratory disease with potential fatal outcome as exemplified by the pandemic-causing SARS- or MERS-CoVs. Despite the frequency and severity of RSV and CoV diseases, attempts to develop an effective and non-toxic antiviral treatment or a vaccine have so far been unsuccessful.

The aim of this thesis was to identify new antiviral candidates for treatment of RSV and CoV infections, and to elucidate their antiviral mechanism. Through screening of the ChemBioNet collection of ~17000 diverse compounds and a mini-library of polysulfated tetra- and pentasaccharide glycosides in a cell culture-based whole virus system, three promising anti-RSV and one anti-CoV candidate drugs were identified. Subsequent application of our step-by-step assay strategy for elucidation of mode-of-antiviral activity (**paper III**), revealed that anti-RSV P13 and C15 compounds displayed potent antiviral activity by targeting the heptad repeat regions of the viral F-protein essential for the virus-cell and the cell-cell membrane fusion (**paper I**). The anti-RSV lead drug PG545, identified in **paper II**, was prepared by coupling of a lipophilic cholestanol group to the synthetic sulfated oligosaccharide. This modification of the oligosaccharide enhanced the anti-RSV activity and conferred virucidal properties on PG545, a feature absent in native sulfated oligosaccharide inhibitors (**paper II**). PG545 exhibited dual antiviral mechanisms including (i) reduction of the RSV attachment to cells due to targeting of the highly conserved region and the receptor-binding region of the viral attachment G-protein, and (ii) direct inactivation of viral particles. The anti-CoV candidate drug K22 potently inhibited 229E-CoV infectivity by targeting the membrane-bound viral RNA synthesis in the cytoplasm (**paper IV**). Analysis of viral variants resistant to K22 in addition to the preparation of specific 229E-recombinant viruses, revealed that K22 targets the viral nonstructural protein 6 (nsp6). This protein is involved in the recruitment and modification of host cellular membranes to create sites for the virus membrane-bound RNA synthesis. This is the first report of nsp6 as a druggable target for CoV intervention. K22 was also shown to be active against many other CoVs including the newly emerged MERS-CoV. In conclusion, P13, C15, PG545, and K22 are promising candidates for further development as new anti-RSV and anti-CoV drugs.

**Keywords:** antivirals, respiratory syncytial virus, coronavirus, antiviral screening, fusion inhibitors, sulfated oligosaccharides, nsp6, membrane vesicles

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