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Opponent's review of the doctoral thesis by Mgr. Kateřina Radilová entitled

„Structural characterization of influenza A polymerase PA subunit domains in complex with novel inhibitors"

The doctoral thesis presented by Mgr. Kateřina Radilová focuses on the development of inhibitors of influenza virus RNA-dependent RNA polymerase (RdRp) as potential antiviral agents. Influenza viruses regularly cause annual epidemics with large numbers of deaths and significant economic consequences. Considerable attention has been paid to the development of antivirals targeting influenza viruses, and therefore I consider the aim of this thesis to be very timely and important. The dissertation is written in a shortened form and has a standard structure. In the literature review (chapter Introduction) the author briefly describes basic facts about influenza, influenza viruses, the structure and life cycle of the IAV virus. The structure of RNA-dependent RNA polymerase (RdRp) and the mechanism of IAV genome replication are then discussed in more detail. In the last part of this chapter, the author briefly summarizes the currently available agents for the treatment of influenza. The final part of the thesis is based on four publications in high quality international journals with IF. Mgr. Hradilová is the first author on one of these publications and on the other two she is the second author with joint first authorship. The author's contribution is described in detail for all publications.

The compounds described in the thesis target two regions of the PA subunit of RdRp. The first site is a key part of its interaction surface with the PB1 subunit. The interaction between PA and PB1 is mediated by a short 3_{10}-helix from the N-terminus of PB1, and it has recently been shown that this interaction can be blocked by a peptide that mimics this part of PB1. In the publication **I**, Mgr. Radilová identified the minimal sequence ensuring binding of this peptide to the PA subunit. The sequence of this decapeptide was then successfully modified to increase binding affinity. The crystal structure of the complex of the PA subunit with the mutated peptide subsequently showed that it binds in a very similar manner as the WT peptide. Publication **II** focuses on the thermodynamic and structural characterization of optimized peptide inhibitors. This work also included demonstration of the function of these optimized peptides in cell culture. In the third publication, the author and her colleagues studied the anti-influenza effects of flavonoids and showed that the mechanism of action of these compounds is based on their interaction with the PA subunit of RdRp. The last

publication focuses on the synthesis and characterization of derivatives of flavonoid compounds identified in Publication **III** as promising RdRp inhibitors.

The formal and graphic quality of the submitted dissertation is excellent. The quality of the English, at least as far as I can judge, is very good.

For the sake of discussion during the defense of the thesis, I have following questions related to presented thesis:

1. How conserved is the interaction interface between PA and PB1 subunits of RdRp? Do you think that the compounds described in your thesis will also inhibit RdRp from other variants of influenza virus (IBV or ICV)?
2. Peptides usually have low membrane permeability. Can the membrane permeability of peptides be increased by other means than adding a cationic CPP(TAT) sequence as used in Publication I?
3. Another problem with peptide-based drugs is the short half-life in the bloodstream due to proteolytic cleavage. How long this half-life should be for a given peptide to be considered promising?
4. How crucial is the presence of 3_{10}-helix in the PB1-based peptide inhibitors? Have you tried to design/prepare peptides with extended structure?
5. The active site of NPA endonuclease contains either two Mn^{2+} or one Mn^{2+} and one Mg^{2+} cations. Does substitution of one Mn^{2+} ion for Mg^{2+} have any effect on endonuclease activity? Has a variant without Mn^{2+}, i.e. with two Mg^{2+} ions, ever been observed?

In conclusion, the doctoral thesis presented by Mgr. Kateřina Radilová represents a significant contribution to the development of antiviral drugs for influenza. The thesis is written in intelligible language and obtained results were published in international journals with IF. The presented thesis clearly demonstrates that Mgr. Kateřina Radilová is able of independent scientific work. Since the presented thesis satisfies all requirements for the doctoral thesis **I fully recommend its acceptance**.

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