

Barcelona, May 20th 2021

Evaluation of the Master Thesis of Matej Miláček entitled: “Novel Agonists of the Juvenile Hormone Signaling”, under the supervision of Prof. Marek Jindra.

From formal and scientific points of view the work is excellent. It is well written and very clear to read. The illustrations are excellent and easily interpretable and the figure captions correctly describe the content of the illustrations. The objectives and the rationale for the Master Thesis are clearly stated. The different sections of the work are very well defined and the extension of each section is correct.

The analysis of the different section of the work, as follows:

The **Introduction** contains the information necessary to put the theoretical framework of the work in context and greatly facilitates the understanding of the results presented. It consists of three well-differentiated sections. First, it presents generic information about the Juvenile hormone and explains in detail the molecular characterization of its receptor (Met/gce-Tai), as well as that of the transcription factors responsible for transducing the hormonal signal (Kr-h1 and E93). In the second part, the structural variability of the different juvenile hormones is described in detail, and also it is introduced the concept of insect growth regulators. Finally, in the third part, it is described the methods that have been used in the work to carry out the screening and functional verification of the selected agonists. It is important to note that the description of the pipeline of the screening for JH agonist, including Figure 4, is very useful for the understanding of the work. Regarding this last point, however, I would like to point out that it would have been interesting to describe the functional criteria used to select the 15

JHags out of the 132 compounds that were obtained after screening, as this point is not clear to me.

In summary, the Introduction is accurate, sufficient and very well structured. The **Bibliography** used is correct and provides the necessary background to understand the subject of the work in a clear and concise way.

The four **Objectives** are concise, clear and perfectly stated.

The **Methods** are very well described and contain all the information necessary to fully understand the different techniques used. I want to highlight the great variety of techniques used, including complicated molecular and cellular biology ones, as well as *in vivo* animal manipulations, which undoubtedly increases the quality of the work and improves the training of the candidate.

The **Results** presented in the different sub-sections are very well stated and the corresponding figures are clear and well designed. First, the relative ability of the 15 selected JHags to induce the interaction of the functional JH receptor is analysed by a two-hybrid system. This result is very interesting as it clearly reveals a strong species-specificity of the different JHags. It is of great importance to highlight the use of 7 species of insects spanning different orders, which gives a clear vision of the range of action of these analogues. The activity of the JHags is then analyzed *in vivo* using *Drosophila melanogaster*, *Tribolium castaneum* and *Pyrrhocoris apterus* as models. The parameters that are measured are the viability and the ability to induce the expression of the JH-dependent gene *kr-h1*. These experiments are very well done and the results are clear and very interesting. Regarding this point, I would have liked to have included the analysis of the expression of Kr-h1 by JHags in *P. apterus*, or at least, explain the reason why this analysis has not been carried out. In general, these results show how important it is to check and confirm the effect of the selected analogues *in vivo*. Moreover, the ability of JHag8 to bind *Drosophila* Gce and *Tribolium* Met *in vitro* is confirmed.

Finally, it is analysed the ability of JH to bind to its receptor in hemimetabolous insects. This result is especially interesting as this interaction had only been demonstrated in holometabolous insects to date.

The **Discussion** is complete and perfectly integrates the results into the conceptual framework of the work. In addition, it satisfactorily solves the possible doubts that generate some of the results.

In summary, I want to emphasize that the work is of very high quality and that the results open the field to continue working on the issue raised. For all these reasons, my evaluation of the master thesis is totally satisfactory. In a 1 to 4 range, in which 1 is the best score possible and 4 is a fail, I grade the work with 1.

Sincerely,

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