

## Expert Opinion on Ph.D. Thesis

Ph.D. Candidate: **Mgr. Gabriel Gonzalez**

This doctoral thesis by Gabriel Gonzalez comes from the laboratory years long focusing on development of bioactive substances. I am saying this fact since this thesis obviously makes tangible and valuable contribution to this overall interest. Specifically, the experiments contained in this thesis brought into life a set of model systems, all based on *in vitro* cultured SH-SY5Y line of neuronal cells, for initial testing of substances for their potential neuroprotective effect. This was obviously very new to the laboratory and as such it opened a completely new avenue in its R&D.

The thesis itself is composed in such a way that it first provides detailed introduction to the medical issue that it tackles, neurodegenerative disorders, and then it describes approaches and methods used to obtain the information on the activity of the compounds of the natural origin that have been tested. Overall, both these parts (about 40 pages) are well organized and written, so that the reader is properly prepared for understanding the “results part” of the thesis. The “results part” begins on page 50 and on 5 pages summarizes the contents and major discoveries that are embodied in the five publications, to which the candidate contributed as the first author (2 publications) and coauthor (3 publications). The copies of all these publications, and also book chapter coauthored by the candidate, are attached as Supplementary materials 1 to 6. As the whole, the dissertation thesis is meticulously elaborated, its language is sound and sophisticated, and its individual parts are well balanced. What is very noticeable is the number of cited works (over 400), what clearly speaks for the candidate to study the field very extensively.

Concerning the quality of the research that makes the grounds of this thesis, I may conclude that it is exquisite. The strategies and findings fully conform to the original plan, and are all published in high visibility journals in the field. The significance for science is unquestionable.

I have the following comments and questions:

Page 37 - please, expand the information on currently available *in vitro* models of PD

Page 38 – are there other ways of differentiation of SH-SY5Y cells than by RA ?

Page 45 – the differentiation was for 48 hours – did you measure some molecular markers or just looked at the cell morphology ?

Page 47 – what was the length of time of treatment in cell viability measurements ?

Page 48 – what is the reason for the different lengths of treatments (1, 2, 3 hours) when assaying caspase activation ?

Page 56 – organoids are very specific model to use mainly because of their heterogeneity – do you have any plan how to deal with this fact ?

In conclusion, based on my opinion detailed above, I strongly recommend this thesis to its oral defense and I suggest the candidate to be awarded by the title “Doctor of Philosophy”.

Brno, June 16, 2021

Doc. MVDr. Aleš Hampl, CSc.

