Reviewer opinion on doctoral thesis of MSc. Alejandro Carazo Fernández: Nuclear receptors – new ligands study and importance of the genetic variability

The doctoral thesis has about 30 pages of text where theoretical background is described in detail. Then follows commentary section on attached papers where candidate is a primary author or co-author. Moreover, a mention is given for ongoing research, which has not been published yet. The thesis was written carefully. However, some minor inaccuracies were not fine-tuned.

Here is the list of some of them:

a) Page 1, 18 – The candidate incorrectly uses the terminology of post-transcriptional and post-translational (page 1 – "Histones can be post-transcriptionally modified by different enzymes,.." , page 18 – ".. function of these receptors are post-translational regulation and post-transcriptional modifications.") – It is well accepted that proteins are post-translationally modificated.

b) Page 5 – figure legend says that numbers represent the length of units/amino acids of the NRs. **This should be explained** as these numbers usually represent something different in phylogenetic trees.

c) Page 32 – Last sentence on isoforms of VDR - the candidate should check the literature again since at least three isoforms are known (VDRA, VDRB1 and truncated *FokI*-VDRA variant).

d) Page 38 – The sentence " PB is a well-known CAR indirect ligand." is incorrect. Since LIGAND is a compound which binds to receptor, terminology of indirect ligand is falling behind the meaning.

For the candidate's work I have some questions:

Questions for A.1.

- Could the candidate mention what the plasmatic concentrations of Chrysin and other tested compounds (including phenobarbital) are in humans ?
- How does the candidate explain that Baicalein induced CYP2B6 but there is no apparent nuclear localization of CAR-EGFP after Baicalein treatment (Figure 1B/C)?

Question for A.3.

- How was calculated IC50 for CYP3A4 inhibition? It is not reached 50% of the inhibition at all.
- Is there any biosynthetic pathway in humans where the DCA 3,12-diacetate could be formed?
- How can be formed acetylated metabolites of any compound in general ?

Question for A.5.

- What are plasmatic concentrations of Leflunomide in treated patients?
- What is the quantitative comparison of phenobarbital at level of CYPs mRNAs induction with Leflunomide? Since it is claimed that this compound is indirect activator as phenobarbital, this compound should be used instead of the ligand CITCO.
- What is the effect of PXR ligands on EGFR? Is there a possibility that Leflunomide is a PXR agonist?

At the end it can be stated that the candidate is able to discuss the topic based on his experimental data. I recommend the thesis for defense and after successful defense I suggest award the candidate the degree Ph.D. according to the valid laws of Czech Republic.

In Olomouc 19.5.2017

doc. Ing. Radim Vrzal, Ph.D.