Ph.D. Review Report

PhD thesis **„Nuclear receptors – study of new ligands and influence of gene variability“** by Alejandro Carazo Fernández, MSc.

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1. General description

The thesis is logically structured and well presented. The document is stratified into chapters according requirements for this type of scientific work, and contain all necessary issues: Introduction, Aims, List of publications, enclosed publications, Discussion, Conclusion etc. I should appreciate comprehensive introduction of the problem, continuing to logically formulated aims. Considering topic focusing the mechanisms associated with action of nuclear receptors, the content of introduction is properly supported by pictures describing principles and classifications. Proper critical discussion explained all detected results. Referred citations are mainly from the last ten years. It supports actual relevance of the subject and proper active work of the author with recent sources of information. The thesis otherwise spans over 113 pages including four enclosed original articles published in journals with impact factors within the interval of 2.245-5.589. Mr. Carazo is the first author of 3 of these works. The articles have already been 22times cited and *h-index* of author is 3. This is impressive performance knowing that all these works have been printed over only last two years.

In my opinion, the thesis fulfils all formal requirements at excellent level.

2. Scientific content

The thesis addresses PXR and CAR receptor agonism and associated intracellular signaling. Specific new compound were studied, such as some flavones, acetylated bile acids or quinazoline derivatives in terms of their influence on these nuclear receptors. Presented results demonstrate original information within the area and all have been published in journals with impact factor (see above). The most significant data presented by the author are:

1. The identification of flovones, chrysin, baicalein, baicalin and galangin as an indirect agonists of CAR receptor. They work in a similar way as phenobarbital, i.e. they inhibited the epithelial growth factor receptor with consequent activation of CAR through dephosphorylation.
2. Introduction of LanthaScreen TR-FRET CAR coactivator assay as a cell-free method to identify CAR receptor agonist and inverse agonists.

3. Description of acetylated deoxycholic and cholic acids as potent ligands of pregnane X receptor using a complex set of *in vitro* methods. Very interesting in this way was analysis of human bile, which is highly unavailable material.
4. The identification of 2-(3-Methoxyphenyl)quinazoline derivatives as promising CAR ligands. Importantly, quality and validity of data for humans was confirmed using primary human hepatocytes.

The scientific content of thesis is current and relevant in the context of up-to-date research of PXR and CAR nuclear receptors.

3. Remarks to thesis:

None.

4. Questions:

- Are there any data describing consequences of the PXR and CAR activation for drug metabolizing enzymes and transporting proteins in the intestine and kidneys?
- Are the any metabolic adverse reactions that can be expected during administration of PXR and/or CAR agonists to humans?

Conclusion:

The author of this dissertation thesis has demonstrated the ability to work independently and creatively in the specific field. In support, data obtained during research work were presented in valuable journals with high impact factors. The thesis meets the standard requirements imposed on the dissertation work in the field. I clearly recommend PhD thesis by MSc. Carazo for defence. After successful process, I recommend granting an academic title „Ph.D.“ to Alejandro Carazo Fernández.

Hradec Králové 30. May 2017

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