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Review of the Ph.D. thesis of António José Ribeiro Pombinho entitled „High-throughput screening for the discovery of small molecules".

The Ph.D. thesis of António Ribeiro dealt with the high-throughput testing of a library of more than 20,000 small compounds for their effects on different cell lines. The aims of the study were to develop and perform high-throughput screening assays, to validate and confirm identified hits and to study the mechanisms of action of the active compounds. The strategy followed three main lines and was focused on affecting cell self-renewal, cell differentiation and finally cell death. The author profited from the cutting edge instrumentation in the CZ-OPENSCREEN center in IMG.

For studying the effects of compounds on the cell self-renewal, the luminiscence-based reporter assay was developed and the author identified monensin as an inhibitor of the Wnt pathway and also two compounds as activators of hypoxia pathway. The author also studied molecular mechanisms of action of these compounds.

The studying of effects of compounds on cellular differentiation was complicated by requirements for special instrumentation as fluorescent microscope or flow cytometer, which, on the other hand, offer multiparametric readouts termed "high-content screening". Another difficulty was also instability of available cell lines. Therefore, these experiments are still ongoing.

Finally, the author discovered another active compound, homoharringtonine, efficiently sensitizing TRAIL-mediated cell death of cancer cells. Moreover, compound diphenyleneiodonium chloride was identified as a potent killer of *Leishmania*.

Ph.D. thesis of António Pombinho brings new high quality results in the field of the high-throughput screening/discovery of biologically active compounds. A. Pombinho had to solve diificult problems and he used sophisticated modern techniques. Monensin/Wnt and homoharringtonine/TRAIL projects were successfully published in highly impacted journals and, in each case, António Pombinho is one of the first two authors who contributed equally to the work. For these reasons I believe that the work of A. Pombinho fulfills requirements for a Ph.D. thesis and confirms the skills of the candidate for an independent scientific work. **I recommend the Ph.D. thesis of A. Pombinho for a defense.**

However, the Ph.D. thesis is also characterized by several drawbacks and I have some critical comments and few questions as follows.

In general, the thesis could be better written in terms of its structure and clarity of conclusions. It is not always easy to understand and identify which were major results and outputs.

Starting from the Figure 40, the figures have a wrong numbering.

I fully understand that modern research, and especially development of assays and screening of compounds is a team work and I have no doubts that A. Pombinho has done a substantial and important part of work. However, his specific and concrete contributions in each of projects should be specifically described in the thesis. Please, do it during the defense.

I miss more detailed description of tested libraries. All the individual compounds mentioned in the text should be accompanied by their structures not only by codes.

The list of author's publications presumably related to the thesis also contains J. Med. Chem. paper and related patent on lipophosphonoxins as new antibiotics. However, this study, which is certainly of a high quality, is only marginally mentioned in the thesis (one paragraph at p. 58). I have also found no relationship to the thesis of another paper attached (Artificial Organs). I think that it is not necessary to add every paper published. Two high quality publications with the author at the first place should be sufficient.

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Specific questions:
The selection of tested compounds/libraries is a crucial point in HTS screening. How were the tested compounds selected? Why these and not others?
How are the compounds stored? Solubilized or as solids? What is the solvent?
Are the biological assays (models) used here typical for HTS? What are the general biological strategies in HTS?
Two compounds were identified as agonists of hypoxia pathway through inhibition of prolyl-hydroxylases. Was this inhibition verified/confirmed in vitro with purified enzymes?

In Prague, September 11, 2014,

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