Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Zuzana Kotrbová

Thesis title: Enzymes of Purine Salvage Pathway in *Trypanosoma brucei* and the Trypanocidal Action of Acyclic Nucleoside Phosphonates
Supervisor: RNDr. Alena Zíková, PhD.

Reviewer: Dr. Matthew K. Gould
Reviewer' affiliation: Ludwig-Maximilians-Universität, München, Deutschland

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	1
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		15
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Formal requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)	**48**	**40**

* Choose one
[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Suggestions and questions, to which the student has to answer during the defense:

Q1: In Section 3.7 of the Materials and Methods chapter you describe the Alamar Blue assay in excellent detail. I notice that the incubation time before adding the resazurin was shorter than is usually the case (just 24 hours versus 48-72 hours (Räz, B. et al, 1997, *Acta Tropica*; Bridges, D. et al, 2007, *Molecular Pharmacology*; etc...)). I was wondering what where your considerations for selecting the final protocol used and whether the final data would be materially different than if a more conventional incubation regimen was used?

Q2: In Section 4.1 of the Results chapter you compare the sequences of TbHGPRT/1 & /2, pointing out the differences in the C-terminus that may impact on localization. However, there also appear to be some polymorphisms around amino acid 50 in the alignment in Figure 4.1. Do you think these have any functional significance? Are they located in any predicted conserved domains?

Q3: In Figure 4.9 of the Results chapter where you demonstrate a differential localization of TbHGPRT/1 and TbXPRT, there appears to be a mix up in labeling of the bottom images in both panels. I'm sure it's just an oversight, but could you clarify the annotations to allow the confirmation of the conclusions you draw in the text?

Q4A: In section 4.3.3.1 of the Results chapter you draw the conclusion that TbXPRT may also metabolize hypoxanthine, since the RNAi of TbHGPRT only resulted in a transient growth phenotype when hypoxanthine is the only purine available for the Purine Salvage Pathway. As this degree of promiscuity is unusual for XPRTs, and the qPCR data in Figure 4.18 only shows mRNA levels up to day 4 of induction, would a more logical explanation not be that after day 4, when growth rates begin to return to that of uninduced, the trypanosomes have escaped from TbHGPRT RNAi?

Q4B: Given that the anti-TbHGPRT antibody does not appear to be sensitive enough for detection of the protein in Bloodstream forms and that while qPCR tells us something of the mRNA levels it does not directly measure protein levels, can you think of an alternative strategy to knock-down TbHGPRT and demonstrate it's continued effect on protein levels?

Eventual mistakes, which the students should avoid in the future:

The definitions of the terms IC_{50} and EC_{50} have become very confused in the trypanosome world and at times have been used interchangeably. Some definitions have been agreed upon in

Evaluation of Master thesis:

Enzymes of Purine Salvage Pathway in *Trypanosoma brucei* and the Trypanocidal Action of Acyclic Nucleoside Phosphonates

Referee: Eva Horáková

In this Master thesis, the student Zuzana Kotrbová has carried out studies on two enzymes from the purine salvage pathway in the BF of *Trypanosoma brucei*. In addition, the student performed some studies on putative inhibitors of those enzymes and evaluated their effectiveness against *T. brucei*. The thesis is divided into seven sections, with the regular arrangement of the work. I think that Zuzka together with her supervisor Alena Zíková presented well-developed project, which is particularly strong in result section. So far some experiments are little bit sketchy, but I'm pretty sure that the work will result soon in a decent scientific publication.

Now to the criticism which inevitably comes with me.

1. The work could be written better. I found several typos through the work, which in my opinion could be avoided with today's technologies and more careful reading. Introduction section may be more robust, with up-to-date publications. The review by Berg *et al.*, 2010b was cited way too much, the author could use the primary citations instead.

2. Graphics presented in individual figures, could me more unified, it would look more professional. Zuzka is showing the same scheme for purine salvage pathway in the work twice (introduction and discussion), which has its logic, but perhaps the latter one could reflect author's findings from the work.

3. I am missing the glycosomal targeting signal highlighted in the protein alignment in the section 4.1.

4. I am missing the errors bars in your qPCR experiment (page 42). How many times was the experiment done?

5. How would you explain the discrepancies between the IC50 values for individual compounds in Table 4.2 and 4.3 (e.g. DA-XII-73 0.69 versus 5.4)? Was the same WT cell line used in both experiments?

6. In your work you are questioning the quality of the *Tb*HGPRT antibody, but from the western presented the intensity of the signal seems to be OK. In my opinion it's worth to test the antibody also in the studied knock-downs! Did you try to do so?

General questions to the student:

1. Did you test the ANP on studied knock-downs? What do you think will be the outcome of such an experiment?

2. Are the ANPs and mononucleotides acting in the competitive manner or do they bound to the respective enzyme together?

3. You performed the experiments in the BF only, explain the rationale for it. Do you expect to see the same cytotoxic activity of ANPs also in the PF?

4. Can you speculate about the differential expression of HGPRT in BF versus PF from the metabolic point of view?

5. The data you showed on TbHGPRT/1_V5 cell line, specifically the action of ANPs on overexpressed protein are not very convincing. Can you think about some other way how to find out whether the enzyme is the target for ANPs?

Overall, this thesis undoubtedly meets the criteria for a Master degree and I would like to grade the thesis based on Zuzka's defence.

27.5.2014

Horáková