CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of Biochemical Sciences

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2023

Student:	**Andro Haddad**
Thesis Tutor:	prof. Ing. Vladimír Wsól, Ph.D.
Consultant:	Dr. Anselm Morell Garcia
Opponent:	RNDr. Miloslav Macháček, Ph.D.
Thesis title:	**Isocitrate dehydrogenase 2 inhibitor enasidenib synergizes daunorubicin cytotoxicity by targeting aldo-keto reductase 1C3**

Scope of work, number of 60 pages, 23 figures, 2 tables, 48 citations

Evaluation of the work:

a)	Processing of the theoretical part:	Excellent
b)	The complexity of the methods used:	Excellent
c)	Preparation of the methodological part (clarity, comprehensibility):	Very good
d)	The quality of the experimental data obtained:	Excellent
e)	Processing of results (clarity):	Excellent
f)	Evaluation of results, including statistical analysis:	Excellent
g)	Discussion of results:	Excellent
h)	Clarity, conciseness, and adequacy of conclusions:	Excellent
i)	Meeting the objectives of the work:	Excellent
j)	Quantity and up to date of references:	Excellent
k)	Language level (stylistic and grammatical level):	Excellent
l)	Formal level of the work (text structure, graphic design):	Excellent

I recommend the thesis for recognition as a rigorous thesis ☒

Comments on the evaluation:

Andro Haddad's work is very well written and although the student is not a native speaker, the English is at a very high level with minimal errors. However, the text has occasional minor flaws of a rather typographical nature: mg/m2 (the number should be superscripted); dividing the figure caption into two pages (e.g. Fig. 4 or 19); separating the units from the value on the next line (e.g., p. 19 5 years or 100 µl and 30 min on p. 29); D/L isomerism should not be capitalized but written in small caps; words from a foreign language should be italicized (in vitro on p. 24 or E. coli on p. 26). Also, the spelling of the compound designation using the Greek alphabet tend to be that letter rather than its transliteration (e.g. PGF2alpha vs PGF2α on p. 10 or 3-alpha-hydroxysteroid-DHs vs 3α-hydroxysteroid-DHs on p. 10; elsewhere this is not a problem: e.g. 17β-hydroxysteroid-DH on p. 15 or 6-phosphoglucono-δ-lactone on p. 28). Benzo[alpha]pyrene should be benzo[a]pyrene (as is correctly mentioned in its abbreviated form B[a]P one time, while incorrectly in the second time B[α]P; both forms appear in the same paragraph several times. On pages 9-14, citations are repeated in the next few paragraphs, while somewhere citations are omitted - this should be unified. Ideally one citation after several paragraphs with information from that one

publication. However, drawbacks mentioned above have minimal effect on the overall quality of the whole diploma thesis. The student has adopted a variety of experimental approaches such as WB, working with cell cultures and HPLC; he has obtained many valuable results which he discusses appropriately.Overall, I evaluate the thesis as being of very high quality.

Questions and comments to student:

1) On page 7 you state that AKR1C3 is overexpressed in some metabolic diseases. Can you give an example?

2) On page 26 you state that the distilled water was of commercial origin (Braun - for injections?). Why wasn't ultrapure water from the department's Milli-Q water purification system used? Is there some specific reason for that?

3) Was the DMEM medium (Lonza) supplemented only with FBS?

4)On page 33 in the methods you state that you used transiently transfected HCT116 cells and A549 cells. Can you explain why?

5) How were the concentrations of 10 and 50 μM chosen for the experiments with the different enzymes? (p. 38)

6) Why did you use only 0 μM concentration of ENA for the experiment with HCT116-EV and not the whole scale as with the HCT116-C3?

Evaluation of the thesis: Excellent		**For the defense:** **Recommend**
In Hradec Králové	29. května 2023	signature of the opponent